LEUKAEMIA

Research and Clinical Practice

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By

F. G. J. HAYHOE

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With 12 coloured plates
and 196 black-and-white illustrations

LONDON

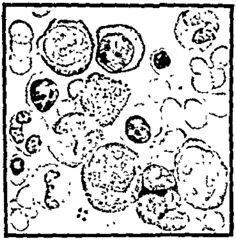
J. & A. CHURCHILL LTD.

104 Gloucester Place, W.1

1960

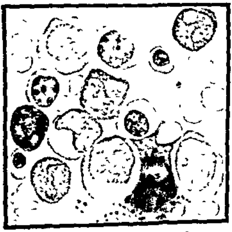
PLATE I

CYTOLOGY AND CYTOCHEMISTRY OF NORMAL HAEMOPOIETIC CELLS



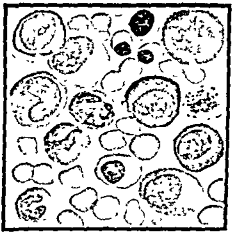
1. May–Grunwald–Giemsa stain

2. Feulgen reaction

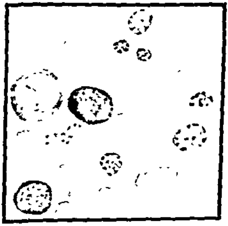


3. Periodic acid-Schiff reaction

4. Sudan black B stain



5. Peroxidase reaction



6. Alkaline phosphatase reaction

Frontispiece

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PREFACE

the generality of neoplastic processes, leukaemia has been of special interest to innumerable workers in the field of cancer research, since the blood and haemopoietic tissues can be so easily and repeatedly sampled. A multiplicity of techniques, physical, chemical and biological, has been directed to elucidating the nature and fundamental components of the leukaemic state, and in recent times more than a thousand articles about the disease have appeared each year in the world-wide scientific literature.

My intention in writing this book has been to provide a guide through the maze of published work, to separate the tangled lines of progress in research, and to give, in small compass, a reasonably comprehensive survey of current beliefs and activities in both the laboratory and clinical aspects of leukaemia.

Such a diversity of disciplines is applied today to different facets of leukaemia investigation that workers in one discipline do not always find it easy to understand the problems and results of those in another. It is, however, imperative that they should do so,

tions of current research within it.

The book contains four chief sections. After a preliminary account of the early history of leukaemia and a short survey of methods used in classifying the disease, the first section is devoted to studies of incidence, ecology and aetiology Statistical data on the mortality from leukaemia in various countries, on the relative frequency of different forms of the disease, and on the influence of age, sex, race, heredity and environmental factors in man are fully reviewed Description and discussion of the very important contributions to our knowledge of leukaemogenesis derived from animal experiments and the more restricted experimental work on transmission of leukaemia in man completes the first section. The second section, in which specific studies of the leukaemic cell are described,

the third section of the book is therefore devoted to a discussion of the general principles which appear to govern the modes of action and usages of currently available therapeutic agents, and which may guide the search for more effective new ones The biological actions of X-rays and radioactive isotopes are considered, and the ways in which chemotherapeutic drugs have been developed and are believed to act are described Common complications of leukaemia, including anaemia, haemorrhage and infections, are also discussed in this section, with regard both to their pathogenesis and to their treatment.

In the fourth section the clinical aspects of each of the different forms of leukaemia

are dealt with in turn, separate chapters being devoted to the acute leukaemias as a group, to chronic granulocytic leukaemia, to chronic lymphocytic leukaemia, and to unusual varieties of leukaemia. In each case the modes of onset, the extent of system involvements and correlated pathology, the blood and bone marrow changes, the differential diagnosis, the most effective methods of treatment, and the course and prognosis are described and discussed. Illustrative case histories are appended.

In the final chapter the nature and nosology of leukaemia are discussed, with a study of the relationships of the leukaemias to leukaemoid reactions and to other myeloproliferative and lymphoproliferative states.

With regard to nomenclature, the terms defined and described in Chapter 2 have been used throughout the book, but certain synonymous alternatives such as lymphatic and lymphocytic, myeloid and granulocytic, have been used interchangeably; they are so well established in common usage that an insistent preference for one form appears pedantic.

The cytological and cytochemical features of leukaemic cells are illustrated both by colour paintings and by half-tone photomicrographs, a combination designed to achieve a high standard of realism In the colour paintings and in certain of the photomicrographs a number of identical fields stained by different techniques have been illustrated, thus aiding the comparison of different staining reactions in individual cells. The thirty-six paintings used in the colour plates have been arranged in two ways; they appear first, grouped according to the staining reaction depicted, in the section on cytology and cytochemistry, and a second time, grouped according to the variety of leukaemic cells shown, in the appropriate sections concerned with the different forms of leukaemia. By this means the appearances of blood and bone marrow preparations from all major forms of leukaemia exposed to a single staining procedure can be seen side by side, while later a range of staining reactions in preparations from each individual form of the disease can be compared.

I am grateful to many friends and colleagues who have helped me, directly or indirectly, in the preparation of the manuscript. To my former teacher, the late Sir Lionel Whitby, I owe a special debt, since he encouraged and inspired my interest in leukaemia during the seven years we worked together, and, indeed, first proposed the writing of this book Among colleagues, past and present, in the University Departments of Medicine, Radiotherapeutics and Pathology and at the Strangeways Institute, are many who have assisted me to clarify ideas by discussion and argument; especially Drs. D. Brinkley, A. Clark, J. H. Crookston, E. Davidson, P. T. Flute, M. Hynes, W. Jacobson, E. M. Kingsley Pillers, D'A. Kok, D. Robertson Smith and H. J. Woodliff, who have worked together with me in the management of many patients with leukaemia, and Dr. A. M. Barrett and his staff, whose scrupulous pathological examinations have been invaluable The Regius Professor of Physic of Cambridge University, Dr. J. S. Mitchell, kindly read the manuscript, and I am most grateful for his helpful comments and suggestions with reference to the chapter on radiotherapy. I thank also the physicians, surgeons, radio-

several retinal photographs for my use and I am most grateful to him for his help. With Drs. D. Gairdner and J. Roscoe, paediatricians to the United Cambridge Hospitals, I have had many helpful discussions during our joint control of children with acute

leukaemia. I should like also to thank the members of the Medical Research Council Working Parties on Leukaemia, including Professors J. V. Dacie and L. J. Witts, Drs. S. T. Callender, W. Davidson, D. A. G. Galton, R. Bodley Scott, and G. Wetherley Mein, as well as other haematologists, too numerous to mention, who have sent me specimens for cytological study. Dr. Joseph Burchenal of the Sloan-Kettering Institute, New York, has been good enough to send me specimens for cytochemical study by air-mail across the Atlantic.

To my present close collaborator, Dr. Dennis Quaglino, I am especially grateful for his skill and care in our current cytochemical researches, some of the results of which have been incorporated in the book, and for his readiness to carry an increasing burden of work while I have been engaged with manuscript and proofs.

I wish to thank the authors, publishers and editors who have allowed me to reproduce illustrations; Dr. I. H. Krakoff, Professor L. J. Witts and the Academic Press Inc. for

the text. I thank also editors, publishers and co-authors who have given me permission to use material from previously published articles of my own, the Editor of the *British Journal of Haematology* and Blackwell Scientific Publications Ltd. for allowing me to quote from articles on 'The management of acute leukaemia in adults' (1955) and 'The cytochemical demonstration and measurement of leucocyte alkaline phosphatase activity in normal and pathological states by a modified azo-dye coupling method' (1958); the Editor of the *British Medical Journal* for permission to quote from 'Medullary aplasia in chronic myeloid leukaemia during busulphan therapy' (1957); and the Editors of the *Quarterly Journal of Medicine* and the Clarendon Press for allowing me to use short extracts from 'Tuberculous miliary necrosis and pancytopenia' (1955)

I am very greatly indebted to my senior technical assistant, Mr. R J. Flemans, who developed and printed all the photomicrographs, supervised or undertook, with the help of Miss S Tomlin, the preparation of the line diagrams, and painted the colour illustrations This last task in particular involved many hours of painstaking and highly skilled work. I am most grateful, also, to my indefatigable secretary, Miss Jean Thompson, who typed and retyped the manuscript and bibliography, often from almost illegible and extensively corrected script.

my appreciation of *Leukaemia Abstracts*, sponsored by the Lenore Schwartz Leukaemia Research Foundation. I have found this periodical of great value in directing attention to publications on leukaemia, especially those appearing in less common journals, which might otherwise have been missed.

I much appreciate the efficient and helpful co-operation of my publishers, J &. A. Churchill, Ltd , especially that of Mr. J. A. Rivers.

Finally, I acknowledge with deep gratitude the unfailing patience and constant encouragement of my wife.

CAMBRIDGE
October 1959

F. G. J. HAYHOE

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CHAPTER 1

HISTORY OF LEUKAEMIA

TOWARDS the middle of the nineteenth century the time became ripe for the recognition of leukaemia. Cases of splenic enlargement accompanied by pallor, purpura, lymphatic glandular swelling and other signs commonly found in leukaemias had been described often since the earliest medical records. A paper entitled "Observations on abdominal tumours and intumescence, illustrated by cases of diseases of the spleen, with remarks on the general pathology of that viscus", from the pen of the eminent Richard Bright of Guy's Hospital, Physician Extraordinary to the Queen, makes clear the position of advanced medical opinion on this subject in 1838 "With regard to the functions of the spleen", he wrote, "we have every reason to believe that it affords important assistance in preparing the blood, but whether chiefly as accessory to the process of digestion, or as having within itself the power of acting beneficially on the blood, I shall not now consider it necessary to inquire It is an established fact, that it is provided with a structure which affords it peculiar elasticity so that it can accommodate itself to great changes in the volume of the blood it contains " Among a large number of "structural alterations" of the spleen categorized by Bright and including congestion, hardening, softening, inflammation, suppuration, gangrene, tuberculosis, malignant disease, melanosis and a form of splenic disease "particularly pointed out by Dr Hodgkin as connected with extensive disease of the absorbent glands" is to be found a condition described as "fleshy hardness with enlargement" Bright noted that "In this state, the spleen often attains to a prodigious size, filling up the whole left side of the abdomen It produces very little constitutional irritation, and chiefly injures by its bulk, and its tendency to favour serous effusion It is astonishing with what rapidity this enormous growth occasionally takes place, but in this [illegible]

in adults, and with them it is more fatal. It often begins to shew itself at two or three months of age, gradually increasing, till it bears a very large proportion to the whole contents of the abdomen, and it is to be traced quite into the pelvis, and extending far beyond the linea alba, towards the right side In these cases, it is often attended with the appearance of petechiae all over their cadaverous and pale bodies Such children seldom live above a year, or two or three, and fall victims to emaciation and often to mesenteric disease " Although Bright included many case reports in this instructive article and one may reasonably interpret several of them as ex[illegible]

concept of splenic involvement in a generalized blood disease may be seen from his

CONTENTS

over, both the clinical and autopsy findings in the cases referred to in the quotation above [illegible] 35) in Donné's [illegible] tedly suffering [illegible] "Un homme, dans la force de l'âge, était atteint d'une artérite qui affectait spécialement les vaisseaux des membres inférieurs; les deux jambes étaient le siége d'ecchymoses, de phlyctènes gangreneuses, etc. Le sang de ce malade présentait une telle quantité de globules blancs, qu'en raison même de la nature de son affection j'étais porté à croire que le sang était réellement mêlé de pus; mais, en définitive, il ne me fut pas possible de constater une difference tranchée entre ces globules et les globules blancs"

In 1845, the year following the publication of Donné's monograph, cases of leukaemia with clinical, post-mortem and microscopic findings were independently and almost simultaneously published in Edinburgh and Berlin. In the *Edinburgh Medical and Surgical Journal* of October 1, 1845, two case reports appeared under the title "Disease and Enlargement of the Spleen in which Death took place from the Presence of purulent matter in the Blood". In the first, David Craigie described a patient who had been under his care in 1841; autopsy, performed by John Reid, revealed "globules of purulent matter" in the blood, great enlargement of the spleen, which weighed 115½ oz. and enlargement of the liver to 99 oz. Mesenteric glands were also noted to be enlarged and the kidneys contained scattered white spots.

It seems likely that neither Craigie nor Reid appreciated the significance of these findings, and the case might not have been recalled or put on record had not Craigie been present at the post-mortem examination of a second, very similar case. An account of this second case immediately follows that of Craigie in the *Edinburgh Journal* of October 1845. The patient had been under the care of Sir Robert Christison, but was described by John Hughes Bennett, who himself performed the autopsy and examined the blood microscopically The patient, a man of 28 years, had complained of increasing lassitude for 20 months and a growing tumour in the left side of the abdomen for 7 months The abdominal mass, painless at first, had become painful during the few weeks preceding his admission to hospital and tender enlarged glands had appeared in the neck, axillae and groins. In hospital he was feverish with a rapid full pulse, developed diarrhoea and died suddenly about 21 months after the first appearance of symptoms

When Bennett carried out the post-mortem examination, 4 days after death, he noted that parts of the blood clot were uncommonly yellow, opaque and dull in appearance and that material resembling thick pus could be squeezed from the cut ends of many veins. The walls of the vessels and heart did not appear diseased There was great enlargement of the spleen, which weighed 124 oz. and had a firm yellow "exudation", an inch deep and three inches long, on its anterior surface The liver was also enormously enlarged, weighing 172 oz. Bennett described these enlargements as "simple hypertrophy". Diseased lymphatic glands, some almost as big as a hen's egg, were found in the inguinal and axillary regions. Glands in the bronchial, mesenteric and lumbar groups were also enlarged and showed on section a greenish-yellow cut surface.

Microscopic study of the blood clot showed the presence of large numbers of colourless corpuscles between an eightieth and a hundred and twentieth of a millimetre in diameter. Bennett, who perhaps first became familiar with the microscopic appearances of blood

penetrating final remarks in this paper. "I may observe generally in reference to splenic disease," he wrote, "that it is probable that the spleen is greatly influenced by the derangement of many of the other organs of the body: . . . for we cannot doubt, that whatever acts decidedly on the circulating system, must, in some degree, influence the spleen; which obviously, from its structure and appearance, receives large quantities of blood, as subsidiary to the processes of sanguification or circulation."

While clinicians were so close to the idea of a disorder of "sanguification" involving the spleen, pathologists and microscopists had been surprisingly slow to exploit and extend the microscopic studies of blood and the recognition of "white globules" initiated by William Hewson in 1774. According to Gowers (1879), "examples of a peculiar alteration in the colour of the blood, suggesting the admixture with it of pus", had been recorded by Bichat and others in the early years of the nineteenth century, and Velpeau in 1827 described a patient with enlargement of the spleen and a rather similar naked-eye appearance of the blood, like the lees of wine, but no microscopic study was carried out and these macroscopic changes cannot be attributed definitely to increased leucocyte content. The same is true of certain earlier records of a milky appearance of blood, such as that of von Haller, quoted by Virchow in 1845

The clear recognition of leukaemia awaited the marriage of clinical and microscopic evidence, and this was brought about independently in several centres within a span of half a dozen years, between 1839 and 1845. Priority is not easy to allocate, since observation in some cases preceded publication by a number of years, while some early observers did not appreciate the significance of their discovery. A somewhat heated controversy on this question of priority mars the early pages of leukaemia's history (see Gowers, 1879; Osler, 1885, Rolleston, 1934; Dreyfus, 1949), but we need not today be unduly concerned with the allocation of credit The discovery was not in any case a stroke of genius; the extensive application of morbid anatomical and microscopic methods to clinical problems had made it inevitable

In France the disease was probably first recognized by Barth and Donné. A woman of 44 years with gross splenic enlargement was admitted to the Hotel Dieu in Paris in 1839, under the care of Barth. At post-mortem examination the blood was found to be semi-purulent, and microscopic study carried out by Donné showed that more than half the

"J'ai plusieurs fois rencontré dans le sang de malades, des proportions considérable de globules ayant tous les caractères des globules de pus, et que j'aurais infailliblement considérés comme tels, si je n'avais pas connu d'une part, la grande analogie de structure et de forme des globules purulents avec les globules blancs du sang, et de l'autre si la nature de la maladie et l'autopsie n'avaient pas éloigné toute idée de pus circulant avec le sang." We may reasonably assume that Barth's case was among the "several" here mentioned, and may perhaps also conclude that Donné had studied the blood of other leukaemic patients, since he is more likely to have noted the very high leucocyte numbers in leukaemia than the moderately increased numbers in inflammatory leucocytoses, and, more-

colourless globules. He was inclined to believe that this disease was induced by exposure to malaria and consisted of a perversion of the nutritive functions, but he argued that

disorder." This is probably the first account of a case recognized in life, but during the next few years diagnosis of leukaemia in the hospital wards became commonplace, as examination of blood under the microscope came to be more often employed in suspected cases of the disease. Gowers (1879) recalled that, in August 1846, W. H. Walshe demonstrated to students in his class at University College Hospital, London, that "colourless corpuscles were as numerous as the coloured discs" in the blood of a patient with enlargement of the spleen. Vogel, in 1849, was apparently the first to make similar observations during life, in Germany, but other records rapidly began to appear in print after this time and an expanding and voluminous literature on leukaemia began to accumulate. John Hughes Bennett published a treatise on leucocythemia in 1852 in which he included a

the ensuing years (1849, 1853, 1864, etc.) Among the many papers, now far too numerous to list, which appeared during these years we may particularly note Biermer's first record of leukaemia in childhood (1861) and Bryant's early unsuccessful attempt to halt the progress of the disease by removing the spleen (1866)

Meanwhile, the conversion of general physicians to the idea of this newly defined disease was gradually occurring, a process well exemplified in the remarks appended by Dr. Samuel Wilks of Guy's Hospital to his report of two further cases diagnosed during life, published in 1855. He wrote "The above cases assist in confirming the observations made by Dr Hughes Bennett respecting the connection of enlargement of the spleen with an excess of white globules in the blood; and they are of the more value because they contrast with a large number of other cases (having no disease of this viscus) where a trial of the blood was made and no leucemic condition found For with the first scepticism which attends the reading of all novel discoveries, there arose the idea of testing the blood in various other cases, where a diseased state of this fluid might reasonably be suspected, in order to ascertain whether only one organ was capable of producing it. The best for this purpose were thought to be all cases of suspicious abdominal tumours, whose con-

have notes of about fifty, and in none, except the two described above, did its condition approach to what could be called leucocythaemia In . . . that class of cases which has especially gained the attention of Dr. Addison, and which he has designated idiopathic anaemia, and where, above all others, it might be presumed that the existence of an excess of colourless globules was probable, no such condition has, as a rule, been found In purpura and scurvy, also, there is no perceptible deviation from the normal relation of the two kinds of corpuscles."

Although in these early years leukaemia was chiefly discovered in association with gross

when he earlier attended lectures given in Paris by Donné, fully described his findings in blood, spleen and glands.

The colourless corpuscles of the blood clot "were round, their cell wall granular, and they presented all the appearance of pus corpuscles. Water caused them to swell and lose their granular appearance, and acetic acid dissolved the cell wall and caused a distinct nucleus to appear. The nucleus was composed sometimes of one large granule about one two-hundredth of a millimetre in diameter, at others of two or three smaller granules, as is seen in the corpuscles of laudable purulent matter.

"The exudation in the spleen was composed of amorphous fibrin mixed with numerous molecules, granular and imperfect cells These were intermingled with bundles of filamentous tissue. The enlarged lumbar glands, on being pressed, exuded a fluid that was crowded with corpuscles, some resembling the colourless corpuscles already alluded to, others oval and round, containing a distinct nucleus"

Neither Craigie nor Bennett regarded the diseases they described as primary disturbances of leucopoiesis. Craigie thought the disorder to be due to chronic inflammation of the spleen and argued that the structure of the spleen was such that pus formed there would not accumulate as an abscess but pass directly into the blood stream. Bennett also thought the colourless corpuscles were pus cells rather than white blood cells and concluded that the disease was a suppuration of the blood

It was left for Virchow in his description of a further similar case, published in November 1845, to recognize the peculiar and individual nature of the disease, to identify the abnormal corpuscles in the blood as white blood cells, and later, in 1847, to propose a new name, "leukaemia", for the disorder. This name did not receive unqualified acceptance, for in a paper read to the *Société de Biologie* in Paris in April 1851, Bennett, after describing and discussing four cases studied personally and a further eight culled from the literature, objected to the term "leukaemia" as a misnomer, since the blood in this disease was not itself white. He proposed "leucocythemia" (λεκὸς, white, κύτος, cell, and αἷμα blood) as a more satisfactory descriptive term. He did, however, accept Virchow's interpretation of the nature of the disease and withdraw his former concept of blood suppuration. We must agree with Gowers, who discussed this problem of terminology in 1879, that "Leucocythemia" would really be a more apt and accurate name, particularly since the white blood corpuscles came to be generally known as "leucocytes" after the first appearance of this word in 1855 in M. P. Littré and C. Robin's Dictionnaire de Médecine (Nysten), but, perhaps because it is shorter, "leukaemia" seems now firmly established.

The cases of leukaemia described by Barth and Donné, Craigie, Bennett and Virchow were all ones in which the blood condition was recognized only *post mortem*. In December 1845 a patient was admitted to St. George's Hospital, London, under the care of Dr. Nairne, with a history of langour and depression for 8 months and a rapidly increasing left hypochondriac tumour. After his death in January 1846, gross splenomegaly and hepatomegaly were found at autopsy and the blood was noted macroscopically to be of a peculiarly grey colour. Henry Fuller, who reported this case to the Royal Medical and Chirurgical Society in June 1846, stated that he had three times during life examined this patient's blood microscopically and again after death, and on each occasion he found, in addition to the natural blood corpuscles, a very large proportion of abnormal, granular,

between the most primitive myeloblast and the fully granular myelocyte to be clearly demonstrated.

The recognition of the myeloblast contributed also to the understanding of acute leukaemic states In 1857 Friedrich had noted the occurrence of a new form of leukaemia with a rapid, acute course, and Gowers (1879) made reference in his review of "splenic leucocythaemia" to "many cases on record in which the symptoms lasted six months only" and some with an even shorter course. He stated, however, that "the most acute cases on record, in which the disease runs its course in a few weeks, are usually attended with great and rapid enlargement of the lymphatic glands and spleen", and since this picture is hardly typical of acute leukaemia as we now know it, the cases may have been examples of acute terminal exacerbation of previously undetected chronic granulocytic leukaemia. The distinction of acute from chronic leukaemia came about gradually; Ebstein, in 1889, described the clinical picture of acute leukaemia on the basis of 16 cases already recorded in the literature, and Fraenkel, in 1895, made a careful study of the blood cells in this condition. Fraenkel assumed that the atypical mononuclear cells he found were early lymphocytes, and he and most of his contemporaries believed that all acute leukaemias were lymphocytic, but he nevertheless concluded that these "lymphocytes" were young forms capable of transforming into polynuclear cells. Ehrlich, writing on "Histology of the blood, normal and pathologic" in Nothnagel's Encyclopaedia of Practical Medicine (English edition, 1905), treats these views of Fraenkel with the greatest contempt. Fraenkel attributed the decrease of polynuclear cells in acute leukaemia to "a disturbance of the conditions necessary for transformation of young forms", that is, to a defect of maturation, and made the difference between acute and chronic leukaemia to be "that in the former the newly formed elements are thrown off from their place of origin into the circulation with such extraordinary rapidity that time is wanting for complete development, while in chronic leukaemia the transition is probably much slower" Ehrlich, who at the time he wrote his article for Nothnagel clearly [illegible]

[illegible] of the blood elements The identification of the myeloblast and the appreciation of its potentialities went far to reconcile the conflicting views, acute leukaemias might be either "lymphogenous" or "myelogenous", and while the majority of peripheral blood leucocytes in either case were non-granular, in the one case their development was restricted to the non-granular lymphocyte line while in the other a tendency, more or less marked, might be shown to develop along the myelocytic chain.

Leukaemias recognized before 1913 were all regarded as examples of disease of the lymphocytic or granulocytic series of cells, and the occurrence of a leukaemic proliferation of monocytes had not been reported, but in that year Reschad and Schilling-Torgau (1913) described a new form of leukaemia involving "splenocytes" or monocytes. The cytological study made by these authors does not appear to have convinced many haematologists of the existence of monocytic leukaemia as a separate entity, for in the next 15 years only six further cases were reported (Clough, 1932), but from about 1930 onwards increasing numbers of reports evidenced the spreading acceptance of the concept

With the definition of a monocytic variety of the disease, the major landmarks in the

splenomegaly, Virchow, in 1847, differentiated two forms of the disease, splenic and lymphatic, and emphasized the importance of lymph node involvement particularly in the latter form. Further major advance had to await the discovery of specific staining methods for blood cells and the rise of haematological cytology, and contributions to the study of leukaemia in the interim tended to be of uncertain interpretation and were often frankly confusing. Thus Cohnheim, in 1865, used the term "pseudoleukaemia" to describe a case with lymphatic glands showing the histological picture of leukaemia but with no significant changes in the peripheral blood. This condition may have been aleukaemic leukaemia, but, since the study of lymph gland pathological histology was still in its infancy, it may equally well have been Hodgkin's disease or some other predominantly lymphomatous disease Again, Neumann's reports of extensive changes in the bone marrow in leukaemia (1870, 1872, 1878), while of fundamental importance, led to difficulties in classification and nomenclature, since he now separated a third category of leukaemia, myelogenous, from the recognized splenic and lymphatic forms.

The introduction of differential staining methods, developed and expanded by Paul Ehrlich from 1877 onwards and described in his monograph "Farbenanalytische Untersuchungen zur Histologie und Klinik des Blutes" (1891), greatly facilitated fresh advance of knowledge about leukaemia as in so many haematological fields. As early as 1879, Ehrlich had published his findings on the specific granules of leucocytes and classified them as eosinophil, neutrophil and basophil. He distinguished all the varieties of normal peripheral blood white cells that we now recognize and made numerous and mainly accur-

leukaemia was essentially identical with the "myelogenous" leukaemia of Neumann. At this stage two major subdivisions of leukaemia were thought to exist, the "splenomyelogenous" form in which large numbers of polymorphonuclear and mononuclear cells containing specific granules were present in the blood, and the lymphatic form in which many non-granular, mononuclear lymphocytic cells were found.

know to be the acute myeloblastic termination of myelogenous leukaemia was also observed and taken to be a transformation from myelogenous to lymphatic leukaemia. The confusion thus brought about began to be clarified when Otto Naegeli, in 1900, recognized and described the myeloblast. The existence of such a non-granular, mononuclear precursor of the granulocyte series of cells provided an explanation for the

hocyte with which it had previously been confused—was assisted by the introduction
lase and peroxidase staining techniques. These methods, modified and applied to
d bone marrow cells by Graham (1916), Goodpasture (1918) and later many others,
d Naegeli's views on the place of the myeloblast by enabling intermediate stages

between the most primitive myeloblast and the fully granular myelocyte to be clearly demonstrated.

The recognition of the myeloblast contributed also to the understanding of acute leukaemic states. In 1857 Friedrich had noted the occurrence of a new form of leukaemia with a rapid, acute course, and Gowers (1879) made reference in his review of "splenic leucocythaemia" to "many cases on record in which the symptoms lasted six months only" and some with an even shorter course. He stated, however, that "the most acute cases on record, in which the disease runs its course in a few weeks, are usually attended with great and rapid enlargement of the lymphatic glands and spleen", and since this picture is hardly typical of acute leukaemia as we now know it, the cases may have been examples of acute terminal exacerbation of previously undetected chronic granulocytic leukaemia. The distinction of acute from chronic leukaemia came about gradually; Ebstein, in 1889, described the clinical picture of acute leukaemia on the basis of 16 cases

leukaemias were lymphocytic, but he nevertheless concluded that these "lymphocytes" were young forms capable of transforming into polynuclear cells. Ehrlich, writing on "Histology of the blood, normal and pathologic" in Nothnagel's Encyclopaedia of Practical Medicine (English edition, 1905), treats these views of Fraenkel with the greatest contempt. Fraenkel attributed the decrease of polynuclear cells in acute leukaemia to "a disturbance of the conditions necessary for transformation of young forms", that is, to a defect of maturation, and made the difference between acute and chronic leukaemia to be "that in the former the newly formed elements are thrown off from their place of origin into the circulation with such extraordinary rapidity that time is wanting for complete development, while in chronic leukaemia the transition is probably much slower" Ehrlich,

it "very difficult to conceive of conditions which would prevent the natural maturing" of the blood elements The identification of the myeloblast and the appreciation of its potentialities went far to reconcile the conflicting views; acute leukaemias might be either "lymphogenous" or "myelogenous", and while the majority of peripheral blood leucocytes in either case were non-granular, in the one case their development was restricted to the non-granular lymphocyte line while in the other a tendency, more or less marked, might be shown to develop along the myelocytic chain.

Leukaemias recognized before 1913 were all regarded as examples of disease of the lymphocytic or granulocytic series of cells, and the occurrence of a leukaemic proliferation of monocytes had not been reported, but in that year Reschad and Schilling-Torgau (1913)

With the definition of a monocytic variety of the disease, the major landmarks in the

panorama of leukaemia had been identified. Controversy over borderline states, interrelationships and innumerable details continued, and indeed is still maintained today, but a general concept of leukaemia had emerged that has not required radical modification in the last 30 years.

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longer than a year, yet it would be misleading to suggest that treatment had converted them from acute to chronic forms of the disease. Again, the onset of chronic leukaemia may be so insidious that the disease can be present for years before the patient seeks medical advice, and when advice is eventually sought a rapid terminal phase may have begun, and death may ensue within weeks. An additional difficulty in applying solely clinical criteria of acuteness is that crises of acute symptomatology sometimes occur during the course of chronic leukaemias and are not necessarily terminal, so that a clinical change from chronic to acute and back to chronic may take place.

Nevertheless, clinical distinctions between acute and chronic forms of leukaemia are broadly paralleled by cytological distinctions and clearly reflect important subdivisions of the disease.

The number of leucocytes in the peripheral blood and the presence of abnormal forms

In both acute and chronic leukaemias the peripheral blood typically contains far more than normal numbers of circulating leucocytes with many immature cells of a kind not normally released from sites of leucopoiesis. This is the classical "*leukaemic*" picture. Such a peripheral blood picture does not, however, always appear, and two major variants are sufficiently common to warrant discussion and a place in any scheme of classification.

They are both states in which the general clinical picture and pathological findings in the bone marrow during life are those of leukaemia, and in which post-mortem examination reveals the characteristic proliferative and infiltrative nature of the disease. In the first condition the leucocyte level in the blood is not raised above the normal upper limit of about 12,000 cells per cu mm., and may indeed be decreased below normal levels, but some of the leucocytes present are immature cells. The name "*subleukaemic leukaemia*" is [illegible] bles the [illegible] moder- [illegible] of the peripheral blood alone would be impossible. The somewhat paradoxical name "*aleukaemic leukaemia*" is applied to this condition.

Subleukaemic and aleukaemic forms are more often encountered in the acute than in the chronic leukaemias, and they usually become fully leukaemic at a later stage in the progress of the disease. They do, however, present difficulties in early diagnosis and their existence emphasizes the importance of bone marrow examination when clinical evidence suggests the presence of leukaemia but blood changes do not conform to the classical pattern

Although the terms subleukaemic and aleukaemic leukaemia are generally restricted to naturally occurring states of the kind described above, they [illegible] accompanied by remission in physical signs and marrow activity.

The identity of the predominating cells and their stage of maturity

Study of the differential cytology of the peripheral blood and bone marrow aspirates offers the most precise means of categorization of leukaemias, since any of the leucocytes

CHAPTER 2

GENERAL CONCEPT AND CLASSIFICATION

THE name leukaemia refers not to a single clear-cut entity, but to a group of disorders in which proliferation, maturation and release of leucocytes and related cells are no longer kept within bounds by the normal physiological mechanisms of control. There is enormously increased activity at sites of leucopoiesis and these sites greatly expand, while large numbers of leucocytes, including immature forms, are often released into the circulation and are found infiltrating many tissues. The disease, untreated, is invariably fatal, and no successful form of curative or permanently controlling therapy has yet been discovered.

Classification

In classifying leukaemias reference may be made to the clinical acuteness of the disease, the number of leucocytes circulating in the peripheral blood and the presence of abnormal forms, the identity of the predominating cells and their stage of maturity, and the site of origin of the proliferating leucocytes In addition, the need for clarity and consistency in nomenclature must be met as far as possible, and this is not altogether easy when cells of wide potentialities and uncertain relationships are involved. Finally, certain "paraleukaemic" states must be discussed.

Acuteness of the disease

A general distinction can be made on clinical grounds between acute and chronic leukaemias. In the former the onset is commonly rapid and the course short and severe, with dramatic symptoms and physical signs of fever, anaemia, haemorrhage, tissue infiltrations, buccal ulceration, secondary infections of respiratory tract, and the like; if the disease is untreated, death usually occurs within 3 months of the onset. In chronic leukaemia, on the other hand, the date of onset is often uncertain, so insidiously do symptoms commence; the condition progresses relatively slowly and may remain mild for long periods with few manifestations of disease other than painless splenomegaly or lymphatic glandular enlargement. Patients with chronic leukaemias nearly always survive more than a year from the time of first symptoms, commonly for 3 to 5 years, and occasionally for very much longer. The survival differences between acute and chronic forms of the disease have been used to provide arbitrary limits, cases of duration less than 3 months (Sturgis, 1955) or 6 months (Custer, 1949) being regarded as acute, those of more than a year's duration being called chronic, while cases of intermediate survival, between 3 or 6 months and 1 year, are described as subacute. Such criteria may be validly applied to many cases of untreated leukaemia, but their general rigid adoption would be quite unsatisfactory for a variety of reasons. Cases with the classical presentation of acute leukaemia, when treated by methods now available, often survive for longer than 6 months and, indeed,

or of irregular nuclear and cytoplasmic outline. When recognizable lymphoblasts dominate the picture, the acute leukaemia may clearly be labelled *lymphoblastic*. The equivalent acute leukaemia of the monocyte series, with monoblasts and early promonocytes in the blood and bone marrow, is separable on morphological grounds into two subdivisions. In the first, a mixed picture of early monocytic and myelocytic cells is seen, and this variety is called acute *myelo-monocytic* leukaemia or the *Naegeli type* of acute leukaemia, since Naegeli (1923) was a strong exponent of the view that monocytes may be derived from the myeloblast or early myelocyte. In the second subdivision the monocytic proliferation is unmixed with granulocytic elements; the picture conforms more to the views of Schilling (1926) that monocytes have an independent origin from the reticulo-endothelial system and is accordingly described as the *Schilling type* of acute leukaemia. When the cells of acute leukaemia show no evidence of differentiation beyond the myeloblast, lymphoblast or monoblast stage, their identification becomes very much a matter of opinion, even when phase-contrast microscopy, supravital staining and histochemistry can be brought to bear on the problem, and many authorities think it best in these circumstances to speak of acute "*stem cell*" leukaemias.

The parallel already instanced between the clinical acuteness of the disease and the degree of anaplasia or lack of differentiation of the predominating cells is also seen in the acute crises occurring during the course of chronic leukaemias, particularly the granulocytic variety. At such a time the mature cells earlier providing the greater part of the peripheral leucocytosis are replaced by primitive cells, and the blood and marrow findings become indistinguishable morphologically from those of acute leukaemia. Subacute forms of leukaemia may also be distinguished cytologically as well as clinically by their possession of a leucocytic pattern intermediate between those of the acute and chronic types of the disease.

The site of origin of the proliferating leucocytes

Very broad subdivisions of leukaemia may be indicated in terms of the site of origin of the affected cells. Thus *myelogenous* or *myeloid* leukaemias are those involving leucocytes normally produced from the bone marrow, while ...
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not find general agreement among haematologists. A second disadvantage of this nomenclature is that it tends to regard the proliferative process, by implication, as confined to the sites of normal origin of the affected leucocytes, whereas extramedullary leukaemic leucopoiesis is commonly prominent in ...
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genous, are commonly employed and serve a useful purpose when a more specific designation is unnecessary or impossible.

normally found in the blood and any of the recognized precursor cells of the leucocytic series may be found to be increased in leukaemias of different kinds. It is generally true that predominance of more mature cells is characteristic of leukaemias with clinically chronic symptomatology and course, while more primitive, less differentiated, cells occur in clinically acute leukaemias.

When the predominating leucocytes are clearly differentiated, as is the case in chronic leukaemias, few difficulties arise in classification. Two chief varieties of chronic leukaemia may be separated on this basis: those with a myelocytic and polymorphonuclear picture, *chronic myelocytic or granulocytic leukaemias*, and those with a mature lymphocytic picture, *chronic lymphocytic leukaemias*. Neutrophil myelocytes, metamyelocytes and polymorphs are typically found in chronic granulocytic leukaemia and the name "neutrophilocytic leukaemia" has been recommended by some authorities (e.g. Forkner, 1938) for this disease. This name offers a sharp distinction between the common form of chronic granulocytic leukaemia and the very rare varieties in which eosinophil or basophil leucocytes and their precursors predominate, but although "*chronic eosinophilic leukaemia*" and "*chronic basophilic leukaemia*" are necessary terms to describe these rare diseases, ordinary granulocytic leukaemia involves not only the neutrophil series, but also, to a less marked extent, both eosinophil and basophil cells, and the more general word "granulocytic" or "myelocytic" appears more appropriate than "neutrophilocytic" or "neutrophilic" in its designation. Comparable subdivisions are, of course, unnecessary in the case of chronic lymphocytic leukaemia, but a difficulty arises from the close cytological similarity of lymphosarcoma cells to mature lymphocytes, and those examples of lymphosarcoma with spill-over into the blood stream may be referred to as a lymphosarcoma cell variety of leukaemia, or as "*leukosarcoma*". While the granulocytic and lymphocytic forms of chronic leukaemia are clearly defined, there is less certainty about the existence and frequency of a third kind of chronic leukaemia, involving the monocytic series. Sinn and Dick (1956) reviewed the small number of adequately documented reports of cases believed to fall into this category and could find only fourteen conforming to their criteria of a duration greater than 12 months or absence of monoblastic proliferation suggestive of an acute leukaemia. They added a further eight cases, but pointed out that "the rarest type of chronic monocytic leukaemia is that in which monocytosis, of considerable degree and consisting of mature cells is present in the blood for long periods of time". Most of their accepted cases had an anaemic or cytopenic onset, remained aleukaemic or subleukaemic for a great part of their course, and only developed frankly monocytic leukaemic changes towards the final stage of the disease. Despite the rather indefinite and non-specific nature of *chronic monocytic leukaemia*, it is convenient to retain the name as a pigeon-hole for the present and to defer more detailed discussion of its possible content until a later chapter.

The cytology of the blood and marrow cells in acute leukaemias lends itself less readily
... since the more immature cells present in the acute disease are
tiation along one or other
yeloblasts and promyelo-
or *myeloblastic-promyelo-*
cytic leukaemia. The terms *micromyeloblastic* and *paramyeloblastic* are sometimes used when the myeloblasts, though recognizable as such, are respectively smaller than usual

variant between a solitary myelomatous nodule and a plasmacytic "leukaemia" with high peripheral plasma cell leucocytosis, while chloroma is a variant of acute myeloblastic leukaemia in which local tumour formation, particularly in the periosteum and bone of the skull, is a striking feature. In this class of malignant paraleukaemic disorders a place should be found for *lymphosarcoma* with its "leukaemic" variant "*leukosarcoma*", already mentioned in connection with lymphocytic leukaemia, and also for *reticulum cell sarcoma* which bears an uncertain relationship to monocytic leukaemia.

Table of classification

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diseases. Subacute forms of disease are not sufficiently sharply differentiated to warrant an elaborate tabulation; they are recognizable only as uncommon states intermediate between acute and chronic leukaemias with variants and related conditions not separately defined from those of their corresponding acute and chronic parallels.

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"Paraleukaemic" states

Under this convenient general heading may be grouped a number of conditions, not specifically leukaemic in nature, but bearing a close relationship to leukaemia. These include other proliferative disorders of haemopoietic cells, such as *polycythaemia vera* and *erythraemic myelosis*, *megakaryocytic myelosis* and *myelofibrosis*, which exhibit a close parallelism to the leucocytic disorder of leukaemia and may develop into partially or even frankly leukaemic states. Certain conditions of *medullary aplasia* must also be included here, since leukaemias sometimes undergo an aplastic change and apparently pure aplasias sometimes become unequivocally leukaemic. Finally, *multiple myeloma* and *chloroma* provide examples of conditions intermediate between unifocal solid tumours and multifocal, generally disseminated, proliferations, since plasma cells may give rise to any

TABLE I *Classification of Leukaemia*

General grouping according to site of origin	*Degree of clinical acuteness and average survival untreated*	*Usual predominating cell type*	*Leukaemic variants*	*Related states*
Myeloid or myelogenous	Acute (less than 6 months)	Myeloblastic	Myeloblastic-promyelocytic Subleukaemic Aleukaemic Micromyeloblastic Paramyeloblastic Myelo-monocytic (Naegeli) Chloromatous Mixed erythro-leukaemia	Acute erythraemic myelosis
	Subacute (6–12 months)	Promyelocytic-myelocytic	No separately defined variants	Subacute erythraemic myelosis
	Chronic (more than 12 months)	Granulocytic myelocytic and polymorphonuclear	Subleukaemic Aleukaemic Eosinophilic Basophilic Acute myeloblastic crisis Leuco-erythroblastic	Myelosclerosis Polycythaemia vera Megakaryocytic myelosis and thrombocythaemia Chronic erythraemic myelosis
Lymphoid or lymphogenous	Acute (less than 6 months)	Lymphoblastic	Lymphoblastic-prolymphocytic Subleukaemic Aleukaemic	Anaplastic lymphoblastic lymphosarcoma
	Subacute (6–12 months)	Prolymphocytic	No separately defined variants or related states	
	Chronic (more than 12 months)	Lymphocytic	Subleukaemic Aleukaemic Lymphosarcoma-cell leukaemia	Lymphosarcoma
Of variable, multiple or uncertain origin	Acute	Undifferentiated stem cells	Subleukaemic Aleukaemic ? Reticulum cell leukaemia	? Reticulum cell sarcoma
	Not generally or clearly separable into acute and chronic forms	Monoblastic-monocytic	Myelo-monocytic (Naegeli) Monocytic (Schilling) Subleukaemic Aleukaemic	Reticulum cell sarcoma ? Histiocytic medullary reticulosis
		Plasmablastic-plasmacytic	Usually subleukaemic or aleukaemic with solitary or multiple plasma cell myeloma tumours Rarely leukaemic	

statistics, while not strictly comparable, strongly suggest that the deaths from leukaemia almost doubled in numbers between 1920 and 1930. With reference to this remarkable rise in mortality from leukaemia, Hewitt (1955) has pointed out that it is proportionately greater than that due to any other disease with the exception of cancer of the lung and coronary thrombosis. Moreover, similar increases have been observed in every country from which statistics are available; figures for the United States, Canada, Denmark, and Scotland, quoted by Court Brown and Doll (1957), provide examples. They are shown in Fig. 2. Other comparable rises have been reported from their respective countries by Lancaster (1955, Australia), Gunz and Hough (1956, New Zealand), Husebye and Gaustad

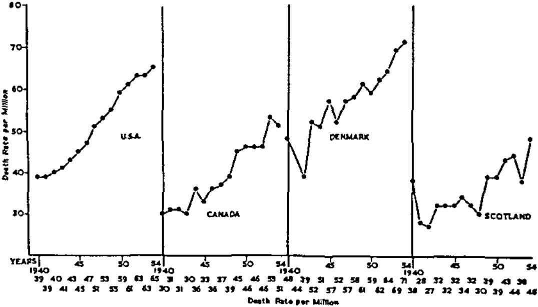

FIG 2. Mortality from leukaemia in various countries, 1940–54

and diagnostic facilities and to the more ready recognition of atypical forms of the disease. These factors may certainly have contributed to the statistical increase, particularly in the earlier years, but it is hard to believe that they can account for more than a small part of the extensive and persistent rise in notifications that has continued without check to the present time

Incidence of leukaemia according to type

It is not possible as yet to obtain adequate data on the incidence of different clinical and cytological types of leukaemia from national statistical records, since the analysis of death certificates for past years does not provide appropriate divisions. The sixth revision of the

CHAPTER 3

THE INCIDENCE AND ECOLOGY OF LEUKAEMIA IN MAN

Vital Statistics

General incidence of the disease

The relatively uncommon incidence of leukaemia and its superficial resemblance to other diseases prevented the accumulation of much informative statistical data in the early years of this century. Before 1920 the International List of the Causes of Death did not separate leukaemia and aleukaemic leukaemia from Hodgkin's disease and "pseudo-leukaemia", and even after this date aleukaemic leukaemia remained classified with

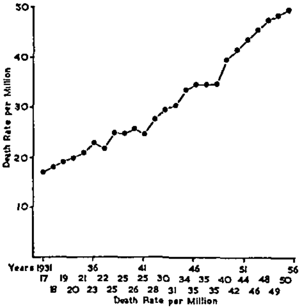

FIG 1. Mortality from leukaemia in England and Wales, 1931–1954

Hodgkin's disease until 1938. Following the 1938 revision a retrospective correction of the figures for England and Wales between the years 1931 and 1939 was made by the Registrar-General (1940) and reasonably comparable figures are therefore available from 1931 onwards of the overall incidence of leukaemia, irrespective of type, in England and Wales. They are given, and depicted graphically, in Fig. 1. The sharply rising trend since 1931 almost certainly continued an existing tendency to increase, for the earlier mortality

statistics, while not strictly comparable, strongly suggest that the deaths from leukaemia almost doubled in numbers between 1920 and 1930. With reference to this remarkable rise in mortality from leukaemia, Hewitt (1955) has pointed out that it is proportionately greater than that due to any other disease with the exception of cancer of the lung and coronary thrombosis. Moreover, similar increases have been observed in every country from which statistics are available, figures for the United States, Canada, Denmark, and Scotland, quoted by Court Brown and Doll (1957), provide examples. They are shown in Fig. 2. Other comparable rises have been reported from their respective countries by Lancaster (1955, Australia), Gunz and Hough (1956, New Zealand), Husebye and Gaustad

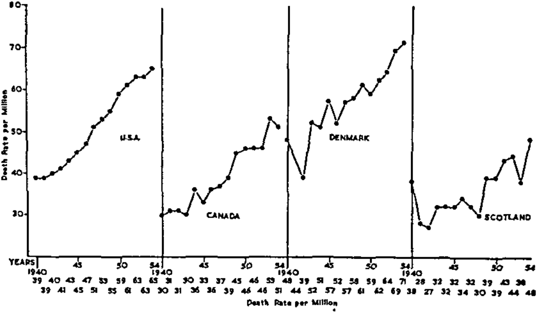

FIG 2 Mortality from leukaemia in various countries, 1940–54

(1956, Norway), Nordenson and Asplund (1956, Sweden), Donner and Maly (1954, Czechoslovakia) and many other authors It has often been argued that the apparent increases in mortality from leukaemia are partly attributable to improvement in medical services and diagnostic facilities and to the more ready recognition of atypical forms of the disease. These factors may certainly have contributed to the statistical increase, particularly in the earlier years, but it is hard to believe that they can account for more than a small part of the extensive and persistent rise in notifications that has continued without check to the present time

Incidence of leukaemia according to type

It is not possible as yet to obtain adequate data on the incidence of different clinical and cytological types of leukaemia from national statistical records, since the analysis of death certificates for past years does not provide appropriate divisions. The sixth revision of the

International List of the Causes of Death (1948) was the first to introduce subdivisions of leukaemia into cytological types, but permitted separation only of "myeloid", "lymphatic" and "monocytic" varieties without reference to acuteness of the disease. On this incomplete basis the figures from England and Wales between
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respectively 6 per cent and 3 per cent of the certifications. A more comprehensive classification is available in the current (7th) revision of the International List (1955), the heading 204, dealing with leukaemia, being subdivided as follows.

204.0—Lymphatic leukaemia.
204.1—Myeloid leukaemia.
204.2—Monocytic leukaemia.
204.3—Acute leukaemia (this will include leukaemia unqualified and any condition in 204.0 and 204.1, if specified as acute, but acute monocytic leukaemia will stay in 204.2).
204.4—Other and unspecified leukaemias.

A further breakdown of acute leukaemias into lymphoblastic and myeloblastic is not allowed for in the List, but this division is being made, when possible, in the United Kingdom, and figures on this more detailed basis are at present being collected by the Registrar-General in this country.

Until national statistics of a more comprehensive kind are published, based on the 7th revision of the International List of the Causes of Death, there are few figures available for the incidence of leukaemia subdivided according to cytology and chronicity and derived from unselected populations Using information extracted by the Registrar-General from the records of all persons certified as dying from leukaemia in England and Wales between 1945 and 1957, Court Brown and Doll (1959) have retrospectively reclassified the cases and calculated separate mortality rates for the acute and chronic forms of the disease The percentage of total leukaemia mortality attributed to each of the chief cytological forms of leukaemia in the adult population as a whole over this period proved to be 19·8 for chronic myeloid leukaemia, 21·2 for chronic lymphatic leukaemia, and 59 for acute leukaemia. The relative frequencies of the cytological varieties of acute leukaemia were myeloblastic 43 per cent, lymphoblastic 21 per cent, and other and unspecified acute leukaemias, many of them presumably monocytic, 36 per cent. The informative survey of Court Brown and Doll, to which further reference will later be made, provides the nearest approach to adequate national data published in this country. Comparable figures from other parts of the world have not yet appeared. There are, of course, many series of collected cases with varying degrees of subdivision into types, derived from hospital or clinic experiences, but such records are from highly selected populations and do not necessarily represent the true incidence of the disease in total populations at risk (Gilliam, 1953). Data from general populations have been collected by Sacks and Seeman (1947) for the City of Baltimore between the years 1939 and 1943, and by Gunz and Hough (1956) for New Zealand between 1950 and 1954, but the first series included only 154 cases (57 incompletely classified) and the second only 553 (30 in-

completely classified). These numbers are too small to allow firm generalizations about the relative frequencies of different types, but it is noteworthy that in each series more than 60 per cent of the classifiable deaths were due to the acute form of the disease, a proportion considerably higher than that found in most hospital series but closely similar to that

TABLE II. *Relative Frequency of the Major Varieties of Leukaemia*

Author	*Period of study*	*Number of cases*	*Chronic myeloid leukaemia (%)*	*Chronic lymphatic leukaemia (%)*	*Acute leukaemia (%)*	*Comment*
Ward (1917)	—	729	34·0	11·5	54·5	—
Rosenthal and Harris (1935)	7 yrs	447	38·0	23·3	38·7	—
Bethell (1943)	15 yrs	399	38·4	19·6	42·0	80 cases of leukosarcoma and 16 of "chronic histiomonocytic" leukaemia are excluded
Gauld *et al.* (1953)	13 yrs	647	26·1	32·0	41·9	—
Best and Limarzi (1952)	26 yrs	892	23·9	31·2	44·9	[illegible]
Gunz and Hough (1956)	5 yrs	523	18·6	15·3	66·1	30 unclassified cases are excluded
Sacks and Seeman (1947)	5 yrs	97	17·5	21·6	60·9	57 incompletely classified cases are excluded
Bernard (1953)	—	1800	28·0	17·0	55·0	—
Guasch (1954) (data collected from 55 centres throughout the world)	—	4705	25·6	22·4	52·0	—

TABLE III *Relative Frequency of Cytological Varieties of Acute Leukaemia*

Author	*Period of survey*	*Number of cases*	*Myeloblastic*	*Lymphoblastic*	*Monocytic*	*Unclassified or "stem cell"*	*Comment*
Rosenthal and Harris (1935)	7 yrs	173	77·4	18·5	4·1	—	—
Bethell (1943)	15 yrs	168	26·2	34·5	39·3	—	—
Gauld *et al* (1953)	13 yrs	271	25·2	53·4	21·4	—	—
Southam *et al* (1951)	23 yrs.	172	38·9	40·1	—	21	Predominance of children in series
Best and Limarzi (1952)	26 yrs	401	37·6	16·3	9·7	36·4	—
Gunz and Hough (1956)	5 yrs	346	38·2	37·6	2·0	22·2	—
Scott (1957)	12 yrs	169	49·7	33·7	13·6	—	Few children in series

given by Court Brown and Doll. The figures are given, together with those from a number of [illegible]

of [illegible]

centres make the figures approximate and not strictly comparable. Certain differences

are sufficiently marked, however, to deserve comment. In Table II the earlier case collections (Ward, 1917; Rosenthal and Harris, 1935; and Bethell, 1943) show chronic myeloid leukaemia to be much more common than the chronic lymphatic form, whereas the later series show a rough equality of incidence. This feature, taken in conjunction with the overall increase in the mortality rate from leukaemia, suggests that the incidence of chronic lymphatic leukaemia is rising more sharply than that of chronic myeloid.

The wide variations in the reported incidence of the different varieties of acute leukaemia shown in Table III are almost certainly due to individual variation in classifying similar material. As Gunz and Hough (1956) point out, it seems unlikely that, with the general uniformity in total incidence of leukaemia over a large part of the world, there can exist such extreme variations in the type distribution, particularly when the most pronounced divergencies are recorded in reports from neighbouring parts of the United States. In the absence of generally accepted and clear-cut criteria for differentiation of acute leukaemic cells, no firm conclusions can be drawn as to the relative incidence of types, and the data collected in Table III serve merely to underline this point.

The incidence of aleukaemic and subleukaemic leukaemia of various kinds is not included in the tables, since information as to its relative frequency in the series listed is not uniformly available Such data as have been published on this matter suggest a fairly high incidence. In the series of Gauld *et al* (1953), 21·6 per cent of the cases had a leucocyte count that did not exceed 10,000 per cu. mm., while among 123 cases of leukaemia encountered by Kirschbaum and Preuss (1943) in a series of 14,400 necropsies, 11 per cent were recorded as "aleukaemic" It is probable that a much higher percentage of cases, particularly acute ones, are aleukaemic or subleukaemic at some stage of the disease.

Incidence of leukaemia in relation to age and sex

Leukaemia occurs slightly more commonly among males than among females. Fig. 3 shows the comparative mortality in England and Wales between 1931 and 1955 and illustrates the persistence of the sex difference A breakdown of the figures according to their classification as myeloid, lymphatic or monocytic can be made from the data available in the national statistics for 1950 onwards The incidence of these varieties of disease according to sex from 1950–53, given by Hewitt (1955) are as follows·

	Per cent male
Lymphatic leukaemia	58
Monocytic leukaemia	54
All types of leukaemia	53
All causes of death	51
Myeloid leukaemia	49

The difference between the sex ratios for lymphatic and myeloid leukaemia is too great to be due to chance or to be attributed to local variations in diagnosis The greater longevity of females would tend to reduce rather than increase the sex ratio of lymphatic as compared with myeloid leukaemia, since there is evidence that the lymphatic form occurs more often at the extremes of life. Hewitt concludes that "there is some aetiological difference between the two main types of leukaemia, which must be associated with a difference between the sexes in exposure or susceptibility to leukemogenic influences".

This conclusion is almost certainly correct, but the figures must be regarded with caution since under both "lymphatic" and "myeloid" leukaemias are grouped unknown numbers of acute leukaemias whose allocation to one or the other cytological division may be open to question Where it is possible to calculate sex ratios from material separated into acute and chronic categories as well as into cytological types, as in the case series of MacMahon and Clark (1956), Shimkin and his colleagues (1951, 1953), Gauld and his colleagues (1953), Gunz and Hough (1956) and Court Brown and Doll (1959), the male predominance is seen to be most marked in chronic lymphatic leukaemia, with about twice the incidence found in females, while in chronic myeloid leukaemia and in acute leukaemia the difference is smaller.

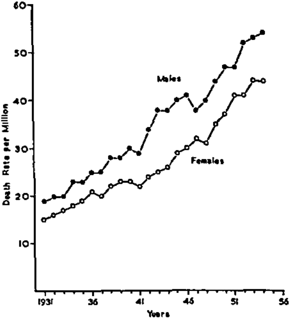

FIG 3 Mortality from leukaemia in the sexes in England and Wales, 1931–54

The general pattern of incidence in either sex and at different ages is not easy to define, since the pattern is undoubtedly changing in parallel with the overall rise in mortality rate from leukaemia. Recent statistical surveys emphasize the conspicuous increase in the incidence of leukaemia in the older as contrasted with the younger age-groups. Sacks and Seeman (1947) noted that the general death-rate from leukaemia increased by 61 per cent between 1931 and 1940, but by 104 per cent in the same period for those aged over 64 years A comparison of death-rates in the United States in 1930–34 with those in 1950–54 by the Metropolitan Life Insurance Company showed a rise of 83 per cent in the mortality from leukaemia among males in age-groups below 45 years but a rise of 183 per cent in those over 45. This sharp age difference was not found among females dying from leukaemia. Cooke (1954) analysed certain features of leukaemia occurrence from the

Vital Statistics of the United States for 1930 to 1949 with special reference to age. The mortality rates in 1930 were compared with those for 1940 and 1949 for each 5-yearly age-group from 0–4 upwards to 75 and over. The figures show small increases in incidence over the 20-year period for age-groups below 50, but above this age the rise in mortality is very striking (Fig. 4). While the relatively small apparent rise in the younger age-groups might be due to better diagnosis, the evidence strongly suggests that a real and sharp increase in the incidence of leukaemia is occurring in persons over 50 years of age.

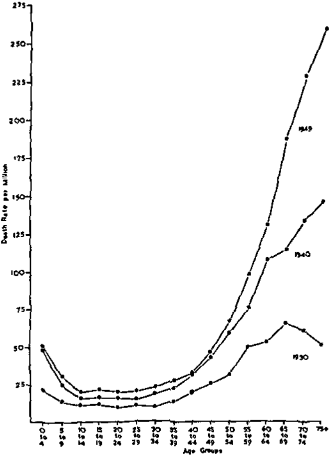

FIG 4 Incidence of leukaemia by age in 1930, 1940 and 1949 (After Cook, J V., 1954.)

An alternative and precisely opposite interpretation of very similar data relating to to the population of England and Wales between 1945 and 1957 has, however, been urged by Court Brown and Doll (1959). These authors point out that improvements in diagnosis and in accuracy of death certification during the period studied have been especially notable in the older age groups and that several causes of death other than leukaemia have shown a comparable large apparent increase, while a large decrease has been observed in the deaths attributed to senility. They therefore conclude that the

apparent increase in mortality from chronic lymphatic leukaemia and a considerable part of that attributed to other forms of leukaemia at ages over 60 are due to better diagnosis, whereas the smaller increases in death rates from acute leukaemia in younger patients are more likely to be real, and perhaps secondary to leukaemogenic factors in the environment. The argument is the more reasonable in that considerable improvements in geriatric services have undoubtedly taken place in Great Britain since 1948. Whether it can be sustained in relation to the elderly populations of other countries, where similar mortality changes have occurred, remains to be seen

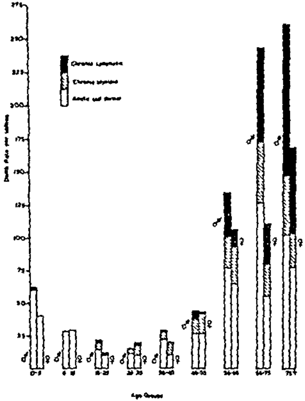

FIG. 5. Incidence of leukaemia by age, sex and type in New Zealand, 1950–54. (From the data of Gunz and Hough, 1956)

Gunz and Hough (1956), studying leukaemia mortality in New Zealand, have shown that here also death-rates rise abruptly after the age of 50 and suggest that this may be a comparatively recent phenomenon. The classification of cases presented little difficulty in this series, over 96 per cent of the total number being unequivocally either acute or chronic, with respective durations of less than 9 months or more than 15 months and having

frequency of the major types of leukaemia in New Zealand according to age and sex, during the years 1949–53. These findings, and those from the studies of Gauld and his associates and of Cooke, are not fully in conformity with past beliefs about the age incidence of leukaemia, nor with statements appearing in several of the current haematological texts. Wintrobe (1956) wrote, "Leukaemia occurs more often in the first five years of life than at almost any other period Until the age of twenty the great majority of cases are acute. From this time until forty-five years of age chronic myelocytic leukaemia is most common. After the age of forty-five the tide turns and chronic lymphocytic leukaemia becomes *the predominating form of leukaemia."* Whitby and Britton (1957) described acute myeloid leukaemia as most common in children and young adults, acute lymphatic leukaemia as having its highest incidence in the first 5 years of life and becoming relatively rare after the age of 25, chronic myeloid leukaemia as most common between 30 and 65, and chronic lymphatic leukaemia as occurring from 35 to 80 with a peak in the sixties Such statements represent widespread clinical impressions and were not clearly contradicted by the statistical data available before 1950 There can no longer be any doubt, however, that these views on leukaemia incidence in relation to age and type require substantial revision. Figs 4 and 5 demonstrate the relatively high incidence of all forms of leukaemia in those over 50, and the data incorporated in Fig. 5 support the view that acute leukaemia is in fact more common among the old than in the young; indeed, the acute form of the disease is the most commonly occurring variety in all age-groups except in those over 75, where it is equalled or slightly exceeded in frequency by chronic lymphatic leukaemia The inferences drawn from these death-rate comparisons are supported by absolute figures of case incidence in several recent surveys. Of 7,593 cases analysed by Cooke (1954) from the U S.A , 19·3 per cent occurred in the 0–14 age-group, 26 5 per cent in the 16–49 age-group and 54·2 per cent in those over 50. The comparable age-group percentage incidences in the 647 cases of Gauld *et al.* (1953) were 17, 34 and 48 respectively, and those in the 553 cases of Gunz and Hough were 20, 22·9 and 57·1.

A general summary of the incidence of leukaemia at present in relation to age, sex and type of disease may be stated as follows. Leukaemia occurs at all ages but is predominantly a disease of the elderly and is least common from 16 to 55. Males are affected more frequently than females at all ages, but the sex difference is small in the middle years, rather more marked in the 0–5 age-group and most conspicuous from 55 onwards. The disparity in sex incidence is least prominent in the case of chronic myeloid leukaemia. Acute leukaemia is the most common variety at all ages, its incidence is low between 16 and 55, higher in children and maximal in the elderly Chronic myeloid leukaemia is rare in children but has a steadily rising incidence with increasing age from 16 onwards Chronic lymphatic leukaemia is rare before 35 but also steadily increases in frequency from this age onwards It is not possible at present to make any substantiated distinctions between the incidence of the different cytological varieties of acute leukaemia, although the disease in children is rarely diagnosed as myeloblastic, being most often described as "undifferentiated" by some authorities and as "lymphoblastic" by others.

Although, as we have seen, leukaemia mortality in early childhood is far less than that in the elderly, the incidence of the acute disease in the first 5 years of life is greater than that in any subsequent age-group until the sixties. Moreover, the distribution of childhood deaths from this cause follows a characteristic pattern During the first 2 years the

incidence is moderately high (about 25 to 30 per million in England and Wales), but in the third and fourth years it rises to about twice the original level. From the fifth to the ninth years a sharp fall occurs, followed by a slower progressive decline to the minimal incidence in middle life. This peak in leukaemia incidence at the ages 3–4 was observed in a series of 1,500 cases collected by Cooke (1942) and is apparent in the national statistics of the United Kingdom. The sharply changing age gradient was regarded as peculiar to leukaemia for some years after it had been noted. Thus Hewitt (1955) could discover no parallel in any other cause of death for which statistics were available, and in particular no parallel in other malignant diseases or in non-neoplastic disorders of the haemopoietic

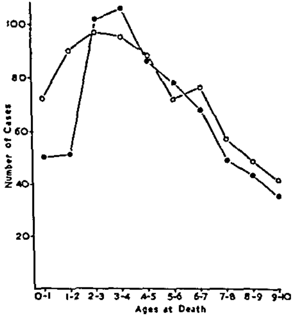

FIG 6 Age incidence of deaths from leukaemia (●—●) and malignant disease (○—○) in children under 10 years (After Witts, 1957)

system. More recent studies, however, suggest that other forms of neoplastic disease in childhood may perhaps have a similar age distribution. Witts (1957*a*) recorded the preliminary results of an investigation by Stewart and her colleagues on the age incidence of 670 cases of leukaemia and 743 of malignant disease in children under 10 years The curves of age incidence, reproduced in Fig 6, are closely similar. The figures, obtained from the Registrar-General, relate to all deaths from these causes in children in England and Wales in 1953 to mid-1955

Racial and geographical incidence of leukaemia

Little is known about the relative incidence of leukaemia among different races and in different areas of the world. National statistics of leukaemia mortality, when they are

frequency of the major types of leukaemia in New Zealand according to age and sex, during the years 1949–53. These findings, and those from the studies of Gauld and his associates and of Cooke, *are not fully in conformity with past beliefs about the age* incidence of leukaemia, nor with statements appearing in several of the current haematological texts. Wintrobe (1956) wrote: "Leukaemia occurs more often in the first five years of life than at almost any other period Until the age of twenty the great majority of cases are acute. From this time until forty-five years of age chronic myelocytic leukaemia is most common. After the age of forty-five the tide turns and chronic lymphocytic leukaemia becomes the predominating form of leukaemia." Whitby and Britton (1957) described acute myeloid leukaemia as most common in children and young adults, acute lymphatic leukaemia as having its highest incidence in the first 5 years of life and becoming relatively rare after the age of 25, chronic myeloid leukaemia as most common between 30 and 65, and chronic lymphatic leukaemia as occurring from 35 to 80 with a peak in the sixties Such statements represent widespread clinical impressions and were not clearly contradicted by the statistical data available before 1950. There can no longer be any doubt, however, that these views on leukaemia incidence in relation to age and type require substantial revision. Figs 4 and 5 demonstrate the relatively high incidence of all forms of leukaemia in those over 50, and the data incorporated in Fig. 5 support the view that acute leukaemia is in fact more common among the old than in the young; indeed, the acute form of the disease is the most commonly occurring variety in all age-groups except in those over 75, where it is equalled or slightly exceeded in frequency by chronic lymphatic leukaemia The inferences drawn from these death-rate comparisons are supported by absolute figures of case incidence in several recent surveys. Of 7,593 cases analysed by Cooke (1954) from the U.S A., 19·3 per cent occurred in the 0–14 age-group, 26·5 per cent in the 16–49 age-group and 54·2 per cent in those over 50. The com-

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to a higher recorded incidence are not clearly determined. Certainly, it is likely that more complete diagnosis will take place and fewer cases of leukaemia be missed, but this explanation can hardly account entirely for the substantial differences existing, since leukaemia is not usually difficult to recognize and is invariably fatal. A second possibility deserving consideration is that a high rate of medical care is itself in some way conducive to the development of leukaemia. The application of X-rays and radioactive isotopes in diagnostic procedures and in treatment and the use of drugs with potential toxicity towards the haemopoietic system provide examples of possible leukaemogenic aspects of medical care.

Close study of leukaemia mortality in the United States has therefore not revealed any undoubted ethnic or racial predisposition to the disease, despite the multiplicity of races making up the population, but a parallel between economic conditions and leukaemia incidence has been established. A similar, though less clear-cut, gradient in incidence between the lower and higher social groups has been demonstrated in the United Kingdom (Hewitt, 1955). Although the matter is at present conjectural, differences in death-rates attributed to leukaemia in various countries of the world may perhaps vary roughly in accordance with the standards of life, and in particular the standard of medical care, existing in those countries [illegible] from those less well-develop[illegible] cidence of the disease might [illegible] 16 countries for 1950–52 has been published by the World Health Organization (1955), but the only non-Western country included in the survey was Japan. It may be significant that here alone among the 16 countries there appeared to be no rise in incidence in the older age-groups, while the overall death-rate was as low as 20 per million

Hereditary influences

The occurrence of such a rare disease as leukaemia in more than one member of a family is a remarkable event, and therefore likely to be reported in the literature. The reviews of Videbaek (1947) and Guasch (1954) show that consanguinous cases of the disease have, in fact, been noted about 150 times, and in some of these instances as many as 4 or 5 members of the same family were affected. The mere existence of so many case reports might suggest that genetic factors were involved, and it is certainly difficult to believe that chance could account for such remarkable familial occurrences as that reported by Anderson (1951), where 5 of 8 siblings developed acute leukaemia in childhood. Nevertheless, statistical genealogical surveys of the families of leukaemic patients compared with suitable control material have not yielded consistent results, and the contribution of heredity to the incidence of leukaemia remains undetermined.

Videbaek (1947) made the first substantial survey of this problem along genealogical lines in Denmark. He obtained information about the incidence of leukaemia, certain other diseases of the blood, endocrine system disorders, and cancer in the parents, grandparents, paternal and maternal uncles and aunts, brothers, sisters, and children of 209 patients with leukaemia. The families of 200 normal subjects were similarly studied for

available and supplemented by figures of the population at risk, can be used to make rough comparisons of death-rates, as has been done in Fig. 2. But such statistics often derive from mixed racial groups, living in widely varying social surroundings and with great differences in availability and use of medical facilities. The difficulties in interpreting data of this kind are well illustrated by the national statistics of the United States with regard to differences in the leukaemia rates in white and negro populations. MacMahon and Koller (1957) used these statistics to compare the mean annual death-rates in whites and non-whites in twenty-two States between the years 1949 and 1953. The disease appeared to be more common in whites in every State, the white to non-white ratio of mortality varying from 2·48 in Georgia to 1·12 in Pennsylvania. The median incomes of white and non-white groups in each State were obtained and the ratio of incomes calculated. For Georgia the figure was again 2·48 and for Pennsylvania 1·42. A striking correlation between leukaemia mortality ratio of white to negro and median income ratio of the two groups was apparent throughout the whole series of States. The authors reasonably conclude that the apparent higher leukaemia death-rate in whites compared with negroes may well be entirely attributable to differences in the "social complex which is closely measured by income, and which includes urbanization, . better medical care and more complete diagnosis". Further, with regard to the importance of the "medical care" contribution to the difference, MacMahon (1957) noted that the overall leukaemia incidence in individual States was more closely paralleled by the number of physicians per 100,000 population in the respective States than by either extent of urbanization or general level of income MacMahon and Koller supplemented their analysis of national statistics by a more detailed study of 1,636 white patients with leukaemia, diagnosed in Brooklyn in 1943–52. These patients were divided into groups according to whether they were native-born or foreign-born, and 1,368 of them were also classified with regard to their religion, whether they were Catholic, Protestant or Jewish. By the use of selected census information for the general population the mortality rate for each of the subdivisions was calculated. A crude comparison between foreign-born and native-born incidence showed a much higher rate in the former group—124·2 per million as compared with 42·7 per million in the latter group—but a considerable part of this difference is due to the greater average age of the immigrant population Nevertheless, when the figures were standardized for age differences a substantially higher incidence in the foreign-born was still found—61 per million as compared with 45·3 per million. It was not possible to make accurate, age-standardized comparisons of leukaemia mortality among immigrants according to their specific countries of origin, but the data available strongly suggested that the higher rate in the group as a whole was attributable to those of Russian birth, who were nearly all Jewish. This conclusion was supported also by the figures of incidence for the different religious groups among the whole population, both native and immigrant. Leukaemia was recorded as cause of death twice as often among Jews as among others, irrespective of country of origin, sex or age. No difference in incidence was observed between other religious groups

The high rate of leukaemia certification in Jews cannot be regarded with certainty as resulting from a genuine ethnic difference in incidence, for there is evidence (Densen, 1956) that Jewish people make more frequent and widespread use of medical facilities than do non-Jewish persons in similar economic circumstances. The factor of availability and use

position to cancer, including a predisposition to leukaemia. The general hereditary predisposition was thought to be present in over 20 per cent of the population and to be transmitted by one or more genes, and gene activity might be responsible for the localization of cancer to the leucopoietic tissue, but external factors probably played an important part in determining the onset of the disease. Leukaemia was more likely to occur at an early age when there was a demonstrable familial tendency and the genetic conditions were particularly favourable, but even with less favourable gene complexes the accumulating effect of external influences might eventually lead to the development of leukaemia and so explain the increasing incidence of the disease in later years

This fundamental study of Videbaek stimulated others to undertake comparable genealogical surveys, despite the formidable difficulties in obtaining accurate information about any but the closest of relatives. Morganti and Cresseri (1954) were able to obtain adequate family details concerning the parents, grandparents, uncles and aunts, brothers and sisters, and children of 476 patients with leukaemia. These patients were selected from a total of 572 leukaemic patients, since family histories could not be obtained for the remainder, but comparison of the 476 chosen cases with the total group showed no selection with regard to variety of disease, sex or age. A control family study of the relatives of 476 healthy persons was carried out to provide data for comparison Among the 6,343 members of the 476 families of leukaemic patients only 3 cases of leukaemia were found; in the control group of 6,321 relatives of 476 healthy persons 2 cases were found These findings, in complete contradiction to those of Videbaek, show no evidence of familial predisposition to the disease. Morganti and Cresseri collected information also on the question of cancer incidence in the two groups of families, and in this respect confirmed the observations of Videbaek by finding a substantially greater incidence among the relatives of leukaemic patients than among the controls. In the former group 233 cancer cases were found in 165 of the 476 families, while in the latter 177 cases in 114 of the 476 families had occurred, thus not only were more families affected in the first group, but the average number of cases in each affected family was higher. The authors concluded that the familial character of leukaemia appeared negligible from a practical point of view, and that the numerous published examples of

Guasch (1954) made a further contribution in this field by collecting information from a large number of haematologists in many parts of the world as to their experience of consanguinous incidence of leukaemia. The response to his enquiries proved difficult to analyse, since many of those who sent data were unable to give the total number of cases studied, and the extent to which family histories had been investigated varied very widely, but a total of 103 examples of family incidence of the disease were reported, excluding those earlier referred to by Videbaek Among the relatives of 8,586 leukaemic patients reported by the 81 haematologists who gave the exact number of their observations, consanguinous leukaemia was found 39 times, giving an incidence of 0·45 per cent While this figure contrasts sharply with Videbaek's 8·1 per cent, the comparison is certainly not valid, since there is no evidence of adequate genealogical investigation having been performed by most of the authors who replied to Guasch's questionnaire. Guasch himself, however, sought information about the incidence of leukaemia and certain other disorders

control purposes. Among 4,041 relatives of the leukaemic patients, 19 cases of leukaemia were reported, 17 of them verifiable, giving a family incidence of 8·1 per cent or more, while in the control group of 3,641 relatives there was only 1 case, giving a control family incidence of 0·5 per cent. Videbaek also found that cancer occurred more frequently in the relatives of patients with leukaemia than in the control group, the percentage incidences being 7·9 and 6 respectively. Calculation of cancer risk (the liability to develop cancer at any age) in the two classes showed an even greater disparity, the estimated risk being 31 per cent in the former and 22 per cent in the latter. From these observations, from more detailed statistical analysis of the leukaemia figures with respect to age, sex and type, and from the results of the survey concerning other blood diseases and disorders of the endocrine glands, Videbaek drew the following conclusions.

1. The occurrence of several cases of leukaemia in the same family was much too frequent to be due to coincidence The observed occurrence of familial leukaemia in over 8 per cent of all cases could not be reasonably explained except on a genetic basis. Simple dominance or recessiveness could be excluded, but transmission might be due to failing dominance of a single gene or to involvement of several genes (polymeria). There was no evidence of sex-linked inheritance and no sex-limitation. There was a significant tendency for chronic lymphocytic leukaemia to become manifest earlier in familial cases than in others, and there was evidence, though less convincing, that the same might be true of chronic myeloid leukaemia and acute leukaemia When the disease affected siblings the onset tended to occur at about the same age, and a less marked correlation of this kind existed between the ages of onset in more distant relatives. The incidence of the various types of leukaemia in the familial cases was the same as that in non-familial cases, and different types of leukaemia were encountered within individual families, so that the genetic factors were clearly not specific with regard to type.

2. The incidence of blood diseases other than leukaemia in the families of leukaemic patients was not abnormally high and no genetic relation was suggested, except in the case of pernicious anaemia, where a definite relation was shown to exist. Among the families of the 209 leukaemic probands pernicious anaemia was found in 17, that is in 8 per cent, while in the control material the disease occurred in only 6 of the 200 families, that is in 3 per cent The difference is statistically significant. Pernicious anaemia was found in familial association with leukaemias of every type and there was no preponderant relation with any one form of the disease

3 The survey produced no definite evidence of a familial association between leukaemia and either diabetes mellitus or disorders of the thyroid gland.

4. The data obtained as regards cancer incidence in the families of leukaemic patients compared with that in the control group demonstrated the existence of a relation between leukaemia and cancer, in that relatives of leukaemic patients developed cancer more frequently than normally. The relatively high incidence of cancer among these families was not attributable to any particular form of localized tumour, but represented an excessive incidence of all forms of the disease.

5. The results of the investigation as a whole supported the concept of leukaemia as a malignant neoplasm, not essentially different from other cancers except in so far as the haemopoietic tissue and the blood occupy a uniquely widespread position in the organism. If leukaemia were so regarded, there might be said to exist a general hereditary predis-

position to cancer, including a predisposition to leukaemia. The general hereditary predisposition was thought to be present in over 20 per cent of the population and to be transmitted by one or more genes, and gene activity might be responsible for the localization of cancer to the leucopoietic tissue, but external factors probably played an important part in determining the onset of the disease. Leukaemia was more likely to occur at an early age when there was a demonstrable familial tendency and the genetic conditions were particularly favourable, but even with less favourable gene complexes the accumulating effect of external influences might eventually lead to the development of leukaemia and so explain the increasing incidence of the disease in later years.

This fundamental study of Videbaek stimulated others to undertake comparable genealogical surveys, despite the formidable difficulties in obtaining accurate information about any but the closest of relatives. Morganti and Cresseri (1954) were able to obtain adequate family details concerning the parents, grandparents, uncles and aunts, brothers and sisters, and children of 476 patients with leukaemia. These patients were selected from a total of 572 leukaemic patients, since family histories could not be obtained for the remainder, but comparison of the 476 chosen cases with the total group showed no selection with regard to variety of disease, sex or age. A control family study of the relatives of 476 healthy persons was carried out to provide data for comparison. Among the 6,343 members of the 476 families of leukaemic patients only 3 cases of leukaemia were found; in the control group of 6,321 relatives of 476 healthy persons 2 cases were found. These findings, in complete contradiction to those of Videbaek, show no evidence of familial predisposition to the disease. Morganti and Cresseri collected information also on the question of cancer incidence in the two groups of families, and in this respect confirmed the observations of Videbaek by finding a substantially greater incidence among the relatives of leukaemic patients than among the controls. In the former group 233 cancer cases were found in 165 of the 476 families, while in the latter 177 cases in 114 of the 476 families had occurred, thus not only were more families affected in the first group, but the average number of cases in each affected family was higher. The authors concluded that the familial character of leukaemia appeared negligible from a practical point of view, and that the numerous published examples of consanguinous occurrence gave an erroneous impression of its frequency, but that there was a real increase in cancer incidence among the relatives of leukaemic patients.

Guasch (1954) made a further contribution in this field by collecting information from a large number of haematologists in many parts of the world as to their experience of consanguinous incidence of leukaemia. The response to his enquiries proved difficult to analyse, since many of those who sent data were unable to give the total number of cases studied, and the extent to which family histories had been investigated varied very widely, but a total of 103 examples of family incidence of the disease were reported, excluding those earlier referred to by Videbaek. Among the relatives of 8,586 leukaemic patients reported by the 81 haematologists who gave the exact number of their observations, consanguinous leukaemia was found 39 times, giving an incidence of 0·45 per cent. While this figure contrasts sharply with Videbaek's 8·1 per cent, the comparison is certainly not valid, since there is no evidence of adequate genealogical investigation having been performed by most of the authors who replied to Guasch's questionnaire. Guasch himself, however, sought information about the incidence of leukaemia and certain other disorders

in the families of 200 leukaemic patients. Among the members of these families, totalling 2,290 individuals, he found only 1 case of leukaemia, 1 of pernicious anaemia, 6 other blood dyscrasias, and 95 neoplastic states (4·1 per cent). In a control series of 4,340 relatives of 375 healthy persons he found 4 cases of leukaemia, 2 of pernicious anaemia, 2 other blood diseases, and 170 neoplastic states (3·9 per cent). Since the average size of the families of his leukaemic subjects was considerably smaller than that of the control series, a direct comparison might be misleading, but with this reservation, the results seem to provide no evidence of a greater frequency of leukaemia, pernicious anaemia or cancer among the relatives of leukaemic patients.

The available evidence on the hereditary background of leukaemia is obviously too contradictory to allow any valid conclusion to be drawn. Future large-scale surveys may decide whether the relatives of leukaemic patients have an increased liability to leukaemia, pernicious anaemia and cancer (cf. Videbaek), to cancer only (cf. Morganti and Cresseri) or to none of these diseases (cf. Guasch). But social and environmental factors may play an indirect part in influencing the results of such surveys, since it is not unreasonable to suppose that members of the same family may tend, within limits, to enjoy a roughly comparable standard of life, and in particular of medical care, and if certain aspects of medical care may contribute, as seems likely, to the development of leukaemia in an individual, they may similarly influence the incidence of leukaemia in his family. It is possible that variations in the importance of external factors of this kind in different countries may explain some of the differences between the conflicting figures from Denmark, Italy and Spain.

The examination of twins has often proved a most valuable method in human genetic studies, but the number of observed and reported examples of leukaemia in one or both of a pair of twins is far too small to provide material for useful analysis Guasch (1954) collected reports of 14 pairs of identical twins and 3 non-identical pairs. Among the former group, in 8 cases both twins were affected at about the same time and in 6 only one was affected during the period of observation, which was from 1 to 2 years in 4 cases and of unspecified duration in the 2 others. Among the non-identical twins, in 2 cases both were affected, and in the third only one, during the observation period of 3 years. It is quite impossible to draw any conclusions as to genetic influence from these records, which are merely an unrelated collection of isolated case reports. The figures suggest that when leukaemia attacks one of a pair of twins the other twin is very likely to develop the disease at the same time; but a strong bias may have been given to these figures, since cases where both twins suffer from the disease are much more likely to excite interest and to be put on record than those where one twin only is affected. Moreover, the extent of exposure to potentially leukaemogenic environmental factors, such as irradiation, is likely to be similar for both of a pair of twins. For these reasons, case reports of the disease in twins have little to contribute to the problem of heredity and leukaemia.

Association of leukaemia with other diseases

The chief diseases which have been linked with leukaemia in the past, because they have occurred together with leukaemia in the same patient or have been found in the same family, are pernicious anaemia and cancer.

In the case of pernicious anaemia, Schumann (1925) first noted the association and

recorded the occurrence of chronic myeloid leukaemia in the daughter of a patient with pernicious anaemia and diabetes, and Koehler (1928) reported the two diseases in siblings. Strandell and Lemming (1931) described two brothers, one with pernicious anaemia and the other with chronic myeloid leukaemia, but rejected most of the cases previously reported in the literature as of uncertain diagnosis. The association of chronic lymphatic leukaemia and pernicious anaemia in the same patient was recorded by Rich and Schiff (1936), and Videbaek (1947) found that 2 of a group of 310 leukaemic patients had suffered from pernicious anaemia for several years before developing respectively chronic myeloid and chronic lymphatic leukaemia. Ardashnikov (1937) found no cases of pernicious anaemia among the relatives of 33 patients with leukaemia, but Werner (1938) found 4 cases of leukaemia among 525 relatives of 57 patients with pernicious anaemia.

The relationship between leukaemia and cancer has also been the subject of many brief reports and the literature contains numerous examples of the coexistence of both diseases in the same patient. Engelbreth-Holm (1941) described 11 cases he had seen and collected 11 others from published reports, and Videbaek (1947) added 2 further cases seen personally and 17 more from the literature. The type of leukaemia involved was most often chronic lymphocytic, but other varieties were found not infrequently No particular kind of malignant neoplasm appeared unduly common in the association. Beresford (1952) found 20 examples of primary malignant disease among 106 patients with leukaemia, giving the very high incidence of 19 per cent. From a comprehensive review of published reports, Moertel and Hagedorn (1957) were able to collect 132 cases of coincident leukaemia and malignant disease, and to these they added a further 52 cases from among 2,134 patients with leukaemia seen at the Mayo Clinic between 1944 and 1953. Of the total number of 184 patients with leukaemia and cancer, 135 had chronic lymphocytic leukaemia, 28 chronic granulocytic leukaemia, 17 acute leukaemia, and 2 an unspecified variety of the disease. This pattern of distribution is to be expected from the predominating age incidence and duration of the varieties of leukaemia, chronic lymphocytic leukaemia occurring in the older age-groups and pursuing a prolonged course. The data from this survey confirmed the conclusions of Videbaek that the presence of leukaemia did not [illegible]

general population, although statistically valid comparisons could not be made in the absence of an adequate control group and in view of the selected nature of the case material.

For the most part these published examples of coincident occurrence of pernicious anaemia or cancer with leukaemia have been isolated case reports or uncontrolled surveys, and do not justify any conclusions as to a genetic or constitutional association between the diseases. The literature has been reviewed by Videbaek and the results of his own more extensive and informative genealogical study have been given above, together with the results of some later and conflicting reports. The important features emerging from all this work are that impressions gained from considering individual case reports may be quite erroneous, that small-scale surveys must be interpreted with reserve, and that large-scale statistical and genealogical studies with adequate controls and due allowance for possible environmental factors are needed before a real association can be accepted or rejected.

In recent years a possible association between leukaemia and Mongolism has been suggested by a number of case reports of the simultaneous occurrence of the two disorders. Dalgaard and Kass (1955) reported a congenital leukaemia in a child with hepatic cirrhosis and Mongoloid facies; Krivit and Good (1956) had seen the association of leuk-

[illegible]

golism seen in a 25-year period. The incidence of 4 in 255 is very much higher than the general incidence of leukaemia, and the authors suggested that the foetal stress causing Mongolism might also injure the developing haemopoietic system and thus predispose to leukaemia, or that undue exposure of Mongols to diagnostic X-rays might be leukaemogenic. In the course of a recent survey of malignant disease in young children, Stewart, Webb and Hewitt (1958) encountered 17 Mongolian idiots among 677 cases of leukaemia, but there did not appear to have been any clear association with a history of irradiation. Excessive maternal age was thought to provide a possible common factor linking the two conditions. These data provide an *a priori* case for further investigation of the relationship, and any future large-scale study should certainly pay particular attention to the possible leukaemogenic role of X-rays, as well as to the question of maternal age.

Radiation and leukaemia incidence

Evidence that leukaemia in man may be induced by exposure to ionizing radiations has become increasingly convincing during the last 15 years. Apart from analogy with the experimental production of leukaemia in irradiated animals, the evidence has been derived from three chief sources. First, the disease appears to be unduly common in radiologists and others occupationally exposed to radiation Second, a marked increase in the death-rate from leukaemia has been observed among the survivors of the atomic bombs dropped on Hiroshima and Nagasaki in August 1945 Third, leukaemia has been noted to develop unexpectedly often in patients treated by radiotherapy, and perhaps diagnostic irradiation may also predispose to the disease.

1. **Leukaemia in radiological workers.** Warren and Dunlap extensively reviewed the literature up to 1942 and found 24 case reports of leukaemia developing in persons chronically exposed to radiation. The 24 victims, 20 men and 4 women, ranged in age from 29 to 53 years, and had all had years of occupational exposure. Several showed radium or X-ray burns of the hands. Four of them had been exposed to radioactive substances and the remainder were radiologists or their assistants and had been working with X-rays The type of leukaemia was described as lymphatic in 7 cases, myeloid in 13, and was unspecified in 4. Once the disease had developed, the subsequent course was like that of spontaneous leukaemia. Among the individual reports summarized above, two are of particular interest, because in each case detailed records were published of the blood picture for a period of years before, during and after the development of leukaemia. Weitz (1938) described the evolution of the leukaemic process in a radiographer whose occupational exposure began in 1928. During 1931 and 1932 her blood picture showed slight relative lymphocytosis, eosinophilia and monocytosis, but a few myelocytes had begun to appear in the peripheral blood in 1933 and at this time the total leucocyte count

had risen to 18,000–20,000 cells per cu. mm. Exposure was stopped at this stage, but a frank myeloid leukaemia was clearly established 9 months later and death occurred in 1938, 4 years after the diagnosis had been made. Maingot, Girard and Bousser (1938) reported a similar sequence of events covering an even longer period. Their patient, a nurse working since 1908 in a radiology department, began to have blood examinations in 1923. Leucocytosis, with an increase in monocytes, was observed to occur sporadically during the next 5 years, but no abnormal cells were seen. From 1928 to 1932, after a short period of marginal leucopenia, a progressive increase in the white count took place, chiefly made up of mature polymorphs and with no immature cells, but a frankly leukaemic picture was found in 1933, with a leucocyte count totalling 53,200 with 13 per cent myelocytes. The patient was still surviving in 1938.

The first statistical evidence that the occurrence of leukaemia in radiation workers constituted a significant occupational risk was presented by Levent (1932), who found leukaemia to be five times as common in physicians practising radiology as in their colleagues. Subsequent statistics on this matter have come principally from the U S A. In 1944, Henshaw and Hawkins calculated that leukaemia was diagnosed 1·7 times as often in physicians as in the general white male population, and Ulrich (1946), from a study of *34,000 obituary notices of American physicians, concluded that the incidence of the* disease among radiologists was more than eight times as high as in other doctors. Further statistical surveys of leukaemia mortality among physicians in general or in radiologists and other specialists have been reported by Dublin and Spiegelman (1947, 1948), March (1944, 1947, 1950), Peller and Pick (1951, 1952), and Warren (1956). Most of the data are not very satisfactory for accurate statistical comparisons with the general population, since elaborate corrections for differences in age distribution have to be made and the

times that among non-radiological physicians These figures of relative incidence do not provide a reliable estimate of risk, and both the extent of diagnostic and therapeutic use of radiation and the standards of protection employed by physicians have changed substantially during the periods of survey, but it would be reasonable to conclude that there has been some increase in incidence of leukaemia among American physicians in the past and that the apparently much higher rate in radiologists follows their occupational exposure to X-rays.

Figures from Britain do not show a similar picture A study by Court Brown and Doll

among those entering radiological practice at a later date These figures are appreciably less than might have been expected from the comparable American data, and the authors suggest that the earlier introduction of protective measures in Britain, the predominant use of X-ray beams of low penetrability and the relatively small number of persons at risk in the early years may explain the lower incidence of leukaemia among British radiologists in the past

2. **Leukaemia in atomic-explosion survivors.** The atomic-bomb explosions over Hiroshima and Nagasaki in 1945 led to a sharply increased death-rate from leukaemia among the irradiated survivors. The increasing incidence was manifest within 3–5 years after the explosions and was first reported by Folley, Borges and Yamawaki in 1952. The results of continued observation and study of the phenomenon, carried out under the auspices of the U.S. Atomic Bomb Casualty Commission, have been published in a series of papers by Moloney and his associates (Lange, Moloney and Yamawaki, 1954; Moloney and Lange, 1954*a*, Moloney and Kastenbaum, 1955; Moloney, 1955), and a summary of data up to August 1955, supplied by the Commission, appeared in a Command paper entitled "The hazards to man of nuclear and allied radiations" published by the Medical Research Council in 1956. From these various reports certain general conclusions can be drawn.

(a) *The extent of the increase.* For the years 1947–54 the leukaemia incidence among survivors varied according to their distance from the hypocentre at the time of exposure. Those who survived the Hiroshima explosion from within 1,000 metres had an incidence about 100 times the calculated expectation among a comparable unexposed population, those from 1,000 to 1,500 metres showed a 22-fold increase, those from 1,500–2,000 metres a 2·6 fold increase, and survivors who had been at greater distances than 2,000 metres from the hypocentre showed only small increases over normal. These rates provide impressive evidence of the leukaemogenic action of a large single dose of ionizing irradiation, but it should be made clear that the total number of cases involved was not great, despite enhanced awareness among diagnosticians of the likelihood of encountering the disease. By the end of August 1955, only 125 cases in all had been discovered among survivors from both Nagasaki and Hiroshima.

(b) *Latent period before onset.* Some cases of leukaemia may have occurred within a year of the bombing (Yamawaki, 1954), but medical facilities and records were so disorganized that collection of adequate data was impossible and the first confirmed cases were recorded in 1947. From this time onwards the annual figures from the two cities showed an incidence rate which rose gradually until 1951 and has since tended to decline, but still remained much above normal 9 years after the bombing. Clearly a latent period of several years may precede onset of the disease after a single massive exposure.

(c) *Dose and type of irradiation involved* The differences in incidence among survivors who had been exposed at different distances from the hypocentre can be very roughly correlated with the falling dosage of irradiation received at increasing distance. Physical estimates of dosage likely to have been received by people standing in the open suggest that, under 1,000 metres, more than 1,400 r of gamma radiation would have been received, at 1,250 metres about 350 r, at 1,750 metres about 50 r, and at 2,000 metres less than 25 r. Neutron flux was estimated to extend only to 800 metres. It is not possible to use these estimates to establish any accurate relationship between dosage and leukaemia incidence, since an unknown number of survivors at each distance must have been protected by various kinds of shielding. Protection would lead to a lower incidence of severe biological effects than would be expected on the official physical estimates, but it seems likely that most of the survivors who later developed leukaemia had been exposed to more than 200 r, judging by their initial degree of radiation sickness. Moloney (1955) pointed out that a further discrepancy of the opposite kind appears to exist between estimated dosages

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(d) *Type of leukaemia induced.* Of 92 cases classified up to January 1954, 52 were acute or subacute, 39 chronic myeloid, and only 1 chronic lymphatic (Moloney, 1955). The

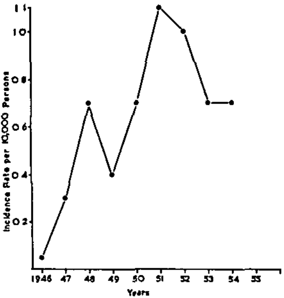

FIG 7 Incidence of leukaemia in survivors from Nagasaki and Hiroshima, 1946–55

extremely low incidence of chronic lymphatic leukaemia makes the distribution of cases very unlike the usual pattern seen in general populations, at least in the West, and it is tempting to account for the relative increase in myeloid leukaemia as due to direct marrow irradiation, perhaps by phosphorus. On the other hand, there is some evidence that chronic lymphatic leukaemia is comparatively rare in Japanese and other Far Eastern peoples (Lange *et al*, 1954, Witts, 1957*b*) and the unusual distribution of leukaemia types among the bomb survivors may be influenced by a factor of inherent susceptibility.

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and observed biological effects, since severe radiation sickness and occurrence of lens opacities due to neutron activity were encountered much further from the hypocentre than would have been expected. It therefore appears possible that neutron activity as well as gamma rays may be involved in leukaemogenesis after the explosions. The neutron effect might be exerted through the prolonged local action of induced radioactive phosphorus, since this isotope has been detected in the bones of persons killed by the bomb (Shimomoto and Unno, 1953).

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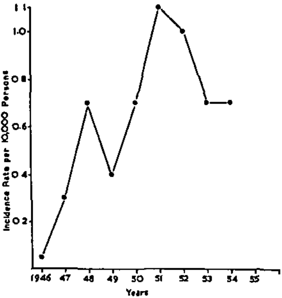

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showed increased susceptibility. Since most young children were evacuated from the cities before the bombing, there were few survivors in the earliest age-groups and no cases of leukaemia below the age of 6. Leukaemia has not developed in any of 33 children who

were *in utero* at the time of the Hiroshima explosion, although 15 of them are mentally retarded (Miller, 1956).

(f) *Early haematological changes.* Moloney and Lange (1954*a* and *b*) described the occurrence of leucocytosis with relative lymphopenia and granulocytosis and the presence of some myelocytes and metamyelocytes in the peripheral blood many months before clinical evidence of leukaemia appeared. Anaemia was not present in these early stages, but there was often a striking increase in basophils and some thrombocytosis. The leucocyte alkaline phosphatase levels were as low as those found in well-advanced cases of chronic myeloid leukaemia. There was no suggestion that either the clinical or the pathological development and manifestations of the disease in the bomb survivors differed from those in non-irradiated subjects

3 **Leukaemia after therapeutic and diagnostic radiation.** From time to time, especially in recent years, isolated case reports and small-scale surveys of patients who developed leukaemia after exposure to radiation therapy have appeared in the literature. The disease has been reported to follow X-ray treatment of non-malignant skin conditions (Gerbis, 1943, Lynch, 1951), thymic enlargement in infancy (Simpson *et al.*, 1955) and ankylosing spondylitis (van Swaay, 1955, Court Brown and Abbatt, 1955, Abbatt and Lea, 1956), and several cases have occurred in patients treated for thyroid diseases with radioactive iodine (Delarue *et al*, 1953, Seidlin *et al.*, 1954, 1955; Blom *et al*, 1955; Abbatt *et al.*, 1956; Pochin *et al*, 1956) Such reports do not, of course, establish that radiation was the effective leukaemogenic agent, since coincidence cannot be excluded in individual cases, but they have directed attention to the possibility that leukaemia may be caused by radiotherapy

Further evidence on the relationship of radiation to the subsequent development of leukaemia has been obtained from an extensive survey of patients treated for ankylosing spondylitis with radiation, sponsored by the Medical Research Council (Court-Brown and Doll, 1957). Among 13,352 patients in the survey, leukaemia was found to have developed in 36. In a further 5 cases the cause of death had been certified as aplastic anaemia, but review of pathological specimens suggested that leukaemia was a more likely diagnosis. Only 1 case of leukaemia occurred among the 2,065 women included in the study, as compared with 40 cases among 11,287 men, and even when incidence rates for men and women were standardized to allow for the tendency for women and older men to have fewer courses of treatment, the rate amongst men appeared to be about three times that in women. The numbers are too small, however, and data about precise dosages too incomplete to justify any certain conclusion as to a sex-difference in susceptibility. The overall incidence rate in the whole group was not high, being less than 3 per 1,000, but calculations from national death-rates showed this incidence to be about ten times greater than would have been expected in a normal unirradiated population of similar age. Examination of a small group of 400 non-irradiated patients with ankylosing spondylitis showed no cases of leukaemia, but this group is too small to exclude the possibility that ankylosing spondylitis may itself predispose slightly to leukaemia, and it has proved difficult to obtain a larger control series since X-ray treatment is so often used in this disease.

Of the 41 cases of leukaemia recorded in the survey, 30 were acute in type, 6 chronic myeloid, 1 chronic lymphatic, and 4 unspecified. Seven of the acute cases were of the monocytic variety. This distribution resembles closely that found among atomic-bomb

survivors and differs sharply from the general pattern of distribution of leukaemia according to type in the non-irradiated population. The length of time intervening between the first course of X-ray therapy and the development of leukaemia was, on average, about 6 years, and this latent period is again roughly comparable to that observed at Hiroshima, although the time measurements are, of course, much less precise, since courses of treatment were often irregularly spaced and covered long periods.

The survey showed a definite relationship between the dosage of radiation and the incidence of leukaemia, although it remains uncertain whether the relation is linear or curvilinear. Few patients were given mean spinal marrow doses of less than 250 r, and the survey has therefore no direct evidence to contribute on possible leukaemogenic effects of doses of this order or less, but for doses from 250 to 2,500 r there appears to have been a sharply rising incidence with increasing dose. An attempt was made to determine whether the extent of fractionation of the total dose received influenced the incidence of leukaemia, but the evidence available was too incomplete to enable any conclusion to be drawn.

Court Brown and Doll noted that a simple proportional relationship existed between dose and leukaemia incidence over the range of dosage observed among patients receiving only spinal irradiation, and they are inclined to believe that a similar relationship may exist at lower dosage levels and that there may be no threshold below which irradiation is no longer leukaemogenic. If this hypothesis were true, any exposure to radiation, however small, would produce some increase in the risk of developing leukaemia, and these authors estimate that the whole marrow dose necessary to double the expected leukaemia risk lies within the range 30–50 r for irradiation of the same energy as used in treating ankylosing spondylitis. The authors are careful to point out, however, that their hypothesis is not the only one compatible with the data, and that a threshold dose may in fact exist.

Diagnostic radiology involves very much smaller doses than those previously considered, but there is evidence that even the small doses, of the order of 2·5 r, involved in pelvimetry may not be devoid of leukaemogenic risk. Stewart *et al.* (1956), Stewart (1957), and Stewart, Webb and Hewitt (1958) compared a number of background factors, including the X-ray histories of mothers of children dying from leukaemia before the age of 10, with those of a control series. An antenatal abdominal X-ray had been carried out in twice as many of the former group as in the controls. The authors have been able to exclude differences in birth-rank and social class and maternal age as associated factors, and conclude that total body irradiation *in utero* by small doses of ionizing radiation probably makes some contribution to the increased leukaemia risk, although other factors such as high maternal age, virus infections during pregnancy, and threatened abortion might also have some association.

Comparable evidence with regard to the possible association between leukaemia in adults and diagnostic irradiation is not yet available, but even if it should prove similar to that shown in children it is unlikely to account for more than a small proportion of the total deaths from leukaemia. Witts (1957*b*) points out the importance of maintaining a proper perspective in balancing the risks of X-ray diagnosis and therapy against their benefits. The leukaemogenic risk of ordinary diagnostic X-rays, if it exists at all in adults, must be extremely small and is far outweighed by the value of the investigation in the

great majority of cases. Heavier irradiation from prolonged screening procedures over the trunk should be reduced as far as possible by improvements in apparatus and exercise of caution. The possible 50 deaths a year which might be attributable in England to diagnostic irradiation *in utero* must be set against the reduction in infant and maternal mortality which may be brought about by appropriate use of X-rays in pregnancy, while the leukaemia incidence of 3 per 1,000 in irradiated patients with ankylosing spondylitis should not obscure the great benefits to be derived from such treatment.

In sum, the contribution of X-radiation to total leukaemia incidence is probably small though not negligible. Since leukaemia following irradiation is most often acute or chronic myeloid in type, this cause is unlikely to be responsible for much of the apparent world-wide increase in spontaneous leukaemia, because a substantial part of this increase is due to the chronic lymphatic form of the disease. The evidence at present does not justify any sharp curtailment of the medical use of X-rays and radioactive isotopes, but the greatest care should be exercised in their use and exposures should be kept to a reasonable minimum.

Chemical agents and leukaemia

The evidence suggesting a leukaemogenic effect of chemical substances used industrially or medicinally is extremely slender in most cases. Occasional isolated case reports have drawn attention to an apparent association between the onset of leukaemia and a history of past exposure to one or other chemical, but the great majority of such associations are probably fortuitous. In the case of benzene, however, and to a lesser extent the sulphonamides and some other potentially myelotoxic drugs, the evidence in favour of their leukaemogenic action is more substantial.

Bernard and Braier (1950) were able to find case reports in the literature of 32 leukaemias attributed to exposure to benzene and added a further 5 cases from their own experience. Since benzene is well recognized to produce marrow aplasia, it seems likely that the true number of leukaemias may be greater, some aleukaemic forms having perhaps been classified as aplastic anaemia The 37 cases collected by Bernard and Braier included 29 males and 8 females, and all the patients except one were adults, mostly of middle age. The single childhood case, described by Mallory, Gall and Brickley (1939), occurred in the son of a painter who had been in the custom of playing with his father's benzene solvent. The remainder all resulted from long periods of occupational exposure, ranging from 8 months to 20 years In some cases the development of leukaemia took place following a period of apparently benign neutrophilic leucocytosis, in others it was preceded by an apparent aplastic state, and in some the blood picture had previously shown no abnormality. Pre-leukaemic blood abnormalities in these patients may of course have been atypical forms of onset of the leukaemia rather than essentially different disease processes. The type of leukaemia observed was chronic myeloid in 5 cases, chronic lymphocytic in 3, and acute or subacute in 29. The general cytological and clinical picture of the disease was the same as that of the comparable type of leukaemia not provoked by benzene, although primitive cells in the acute and subacute forms tended to show rather marked nuclear irregularities of the "paramyeloblastic" kind, and in some cases the medullary leucoblastosis was less intense than is usually found, while extramedullary leucopoiesis

and leucoblastic infiltration were less prominent. The blood and marrow in many cases were shown to contain benzene, and this finding was uniformly positive in every case examined.

A definite cause-and-effect relationship has not been proved beyond dispute by these observations on the association of leukaemia and chronic benzene intoxication, but the predominance of acute forms of the disease and the invariable long period of exposure in the reported cases, together with the high level of benzene in the blood whenever this investigation was performed, strongly suggest a pattern of response to the poison rather than coincidence. No statistical assessment of the leukaemia risk among benzene workers is available, although the risk is certainly not very high or many more cases would undoubtedly have been reported. Rejsek and Rejskova (1955), in the course of a 25-year study, did not encounter a single case among 4,538 persons occupationally exposed to benzene. Animal experiments have also failed to contribute information on the pathogenesis of benzene leukaemia, since, apart from the early work of Lignac (1933) in which very few animals were used, attempts to reproduce the disease in laboratory animals have been unsuccessful (Bernard and Braïer, 1950, Moeschlin, 1951).

Wilkinson has drawn attention to the occurrence of acute leukaemias after sulphonamide therapy (Leonard and Wilkinson, 1955) and has put on record 9 such cases In 2 the acute leukaemia was preceded by sulphonamide agranulocytosis. Other references to a possible leukaemogenic action of sulphonamides, antibiotics and other drugs capable of damaging the bone marrow have appeared from time to time (Hueper, 1942; Marchal, Deprez and Blanc, 1944; Dameshek, 1947, Loyd, 1952, Block, Jacobson and Bethard, 1953; Lebon and Messerschmitt, 1955), but not with sufficient frequency to establish a firm association Most of these drugs are so widely and commonly used, and the development of leukaemia so rare among patients taking them, that any possible leukaemogenic action must certainly be extremely weak, or perhaps operating on a background of predisposition already present in the patient from some other cause.

Blood groups in relation to leukaemia

The genetic relationship suggested by Videbaek (1947) to exist between leukaemia, pernicious anaemia and cancer might be expected to involve an association between

and other blood groups, but no unusual distribution of groups has been observed (Tinney and Watkins, 1941; Best *et al.*, 1949, Lucia and Hunt, 1951, Kay and Shorter, 1956; Buckwalter *et al.*, 1956; Walther *et al*, 1956). This negative finding provides an additional argument against the existence of a pernicious anaemia–leukaemia genetic relationship

The influence of infections and trauma

In a high proportion of cases of acute leukaemia the discovery of the disease is preceded by episodes of infection, varying in site and often severe, and for this reason Cooke (1942), Brown (1951) and others have regarded acute infections, particularly in children, as

probable aetiological factors in leukaemia. The evidence is far from conclusive, and it seems at least equally likely that the infective episodes in question are examples of secondary infections, to which leukaemic patients are prone, occurring during the early stages of the disease. An equal scepticism appears justified concerning the predisposition to leukaemia sometimes attributed to trauma. Many cases have certainly come to light after a traumatic accident, usually a bone fracture or abdominal contusion (Yaguda and Rosenthal, 1939; Olovson, 1939), but evidence of causation is lacking In most cases the leukaemia was probably already existing before the trauma, and in others the connection was remote and tenuous.

Leukaemia and atherosclerosis

A negative correlation has long been observed to exist between atherosclerosis and certain malignant diseases, including the leukaemias Until recently the explanation generally accepted was that weight-loss in malignant diseases would counteract any tendency to lipoid deposition in the arterial wall. Wilens (1947), in support of this theory, found less severe coronary atheroma, with much less lipoid infiltration, in patients who had lost weight during their terminal illness than in those who had not On the other hand, Elkeles (1956), who conducted a radiological survey of the extent of aortic calcification in 400 patients with proven malignant disease, compared with 700 control cases, recorded his impression that the reduced frequency of calcification in the group with cancer was not the result of wasting. His results showed marked differences between the extent and frequency of calcification in malignant conditions arising from different primary sites, deposits being rare in gastric, mammary or prostatic tumours, but as common as among the control group in respiratory tract cancers. Creed and his associates (1955) found from a necropsy study of the aortas of 1,223 male patients with hypertension, bronchogenic carcinoma, Laennec's cirrhosis, or malignant lymphomas, that differences in the severity of atherosclerosis in the various groups were statistically significant. In bronchogenic carcinoma, as in hypertension, there was a greater degree of atherosclerosis than in controls and this increase in severity was more rather than less marked in patients who had rapidly lost weight during the terminal disease. In cirrhosis and in malignant lymphomas including leukaemias the severity of atherosclerosis was definitely decreased In all conditions, prolonged poor nutrition and slow loss of weight over many months was associated with less severe atherosclerosis than was found in patients with the same disease but without weight-loss or with rapid terminal weight-loss of more than 20 pounds in the last 6 months The authors suggest that the small extent of atheroma in leukaemia may be a result of hepatic infiltration, with defective oestrogen inactivation and a consequent prolongation of the clearing action of oestrogens on plasma lipoproteins. The evidence suggests that the negative correlation between atherosclerosis and leukaemia is not solely attributable to the influence of malnutrition and weight-loss. Some other factor is involved. This may be one resulting from the disease process, as Creed and his associates suggest, but in view of the possible genetic relation between leukaemia and cancer, a further genetic association between these diseases and atherosclerosis might perhaps exist (*Lancet*, Annotation, 1957). Appropriate data have not yet been accumulated to allow this hypothesis to be put to a statistical test.

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CHAPTER 4

AETIOLOGY OF LEUKAEMIA

Experimental Studies

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of direct experimental research on aetiology and pathogenesis in man, and the greater part of the information at present available about the causes of human leukaemia has been derived from analyses of mortality from the disease among persons whose circumstances differ fortuitously in one respect or another, as in radiologists compared with other physicians, or from large-scale surveys of populations or families

An immense amount of experimental work has, however, been carried out in animals, especially in poultry and mice, and much is known of the aetiological importance of genetic and constitutional factors, external agents, the endocrine glands and the thymus, while strong evidence now exists in support of a viral cause for the disease in certain species. Experimental data from animal leukaemias cannot, of course, be held to apply directly to the disease in man, but some analogies and parallels unquestionably exist, although, according to Hauschka and Furth (1957), it is doubtful if the precise equivalents of chronic lymphatic or granulocytic leukaemia of man, or acute leukaemia of childhood, have been reproduced in animals However this may be, it is reasonable to expect that continued studies of those animal diseases that are at least roughly comparable to leukaemia in man may produce some increase in our knowledge of the fundamental mechanisms of leukaemogenesis

In addition to systematic studies confined to laboratory animals, experimental work has also been directed to exploring a variety of separate hypotheses, including the possibilities that human leukaemic tissues might be transplantable to other species, that defects of leucocyte elimination from the circulating blood might contribute to the pathogenesis of leukaemia, and that deficiency of some essential metabolite or a disturbance of chemical equilibrium might cause the disease.

Transmissible Avian Leukaemias

Leukaemia occurs spontaneously in many kinds of birds. In his monograph on leukaemia in animals, Engelbreth-Holm (1942) reviewed the earlier literature extensively and noted reports of the disease in turkeys, geese, ducks, pigeons, swans, storks, parrots, parrakeets, canaries and vultures. Experimental research on avian leukaemia has been done almost entirely in domestic fowls, since the disease is common enough in this species to present an economic problem in poultry farming.

Although diseases resembling leukaemia and lymphosarcoma had long been known to affect chickens, the first important discovery about fowl leukaemia was not made until 1908, when Ellerman and Bang succeeded in transmitting the disease from affected to healthy birds by the intravenous or intraperitoneal injection of leukaemic blood or tissue fragments. Transmission could also be achieved with cell-free filtrates. Subsequent work has confirmed the viral aetiology of many, if not all, varieties of fowl leukosis, but the experimental studies reported in the literature are often confusing and difficult to interpret and summarize, since the pathological states under study have frequently been poorly defined and clear discrimination between cell transplantation and virus transmission has not always been made. The tangled literature has been reviewed by Beard, Sharp and Eckert (1955). Two chief forms of autonomous proliferative disease appear to arise from the blood-forming cells of the fowl. The first, lymphomatosis, of common natural occurrence and rapidly spread by contact, consists of a group of closely related disorders involving the lymphocytic series of cells, in which widespread diffuse or nodular infiltrations of the liver, spleen, bone marrow, kidneys and other organs takes place. The disease is aleukaemic in the majority of cases, but a peripheral lymphocytosis is sometimes found (Burmester, 1952). Lymphocytic and plasma cell tumours may occur in association with nerve trunks or in the iris, producing paralytic neuro-lymphomatosis (range paralysis) or grey eye and blindness, these infiltrations are often accompanied by visceral involvement and are probably variants of the general lymphomatous disease caused by different but related viruses. This belief is supported by virus transmission experiments, for, while visceral lymphomatosis has been serially passaged many times in cell-free filtrates, paralytic neuro-lymphomatosis has not occurred with significant frequency, as a complication of the transmitted disease (Burmester, 1947, Davis and Doyle, 1947; Burmester and Gentry, 1954).

An associated neoplastic disease, osteopetrosis gallinarum, arising from cells of periosteal origin and not a tumour of the haemopoietic system, frequently occurs in the course of transmission experiments with some strains of avian visceral lymphomatosis, particularly strain RPL12. Although osteopetrosis arises commonly in birds inoculated with lymphomatous tumour extracts or filtrates, it does not appear to be transmissible by contact, and the causative agent probably differs from that of the associated lymphomatosis (Burmester and Gentry, 1954).

The second form of avian leukaemia, erythromyeloblastosis, is more acute and intensely malignant in course than lymphomatosis, and differs also in being frankly leukaemic or erythraemic, with as many as two or three million primitive cells per cu. mm. in the peripheral blood. This acute disease has long been recognized to be separable into erythroblastosis and myeloblastosis, but combined forms, with apparent involvement of both cell series, have been encountered so often that a clear differentiation between the two variants has seldom been emphasized. The haematological and pathological findings were well described and illustrated by Furth (1931) and may be roughly compared with those in human acute leukaemia and acute erythraemic myelosis. The number of primitive cells

spontaneously or as a result of experimental transmission, or, occasionally, following cell passage from cases of naturally occurring lymphomatosis (Burmester, 1952; Eckert, Beard and Beard, 1953), but the separate aetiological identity of these diseases and of lymphomatosis has become more firmly established in recent years. Cell-free transmission of lymphomatosis, using the RPL 12 strain derived by Olson (1941) from a spontaneous case of the disease and shown to be due to a filtrable agent by Burmester and his associates (1946, 1947), has been continued over very large numbers of passages by several workers without the emergence of any typical erythroblastotic or myeloblastotic forms. Nevertheless, the pathological nature of the transmissible tumour strains of lymphomatosis is not entirely certain. Darcel and Negroni (1954) argued that the transmissible RPL strains were more closely related to erythroblastosis than the term "lymphoid" would suggest, since the tumour cells more closely resembled pro-erythroblasts than abnormal lymphocytes. They suggested that classical visceral lymphomatosis does not contain a viral tumour-inducing agent, thus explaining the frequent failure to achieve transmission of spontaneous lymphomatosis, emphasized by Engelbreth-Holm (1942). The apparently transmissible strains, in their view, might be examples of aleukaemic erythroblastosis with local erythroblastomatous tumour formation. Whatever the outcome of this cytological dispute, the aleukaemic "lymphomatoses" in question differ both pathologically and aetiologically from erythroblastosis and myeloblastosis. The induction of RPL 12 lymphomatosis by cell-free filtrates is characterized by a long latent period, usually over 100 days in 1–3-day-old chicks inoculated intraperitoneally (Burmester and Cottral, 1947), whereas both erythroblastosis and myeloblastosis can be transmitted with very much shorter latent periods. The two latter diseases have been sharply separated in the recent studies of Beard and his co-workers Cytologically, they are, of course, clearly distinguishable when occurring in unmixed form with respectively erythroblastic or myeloblastic peripheral blood pictures. Eckert, Beard and Beard (1956) summarizing the points of difference between the two diseases from the [illegible]

blastosis. Whereas in myeloblastosis successful transfer could only be achieved in chicks inoculated during the first few days of life, age at time of inoculation exerted very little influence on host susceptibility to erythroblastosis virus. Morphologically, however, the viruses of these two diseases appear identical in electron micrographs (Sharp, Beard and Beard, 1955). They are spheroidal, of average diameter 120 mμ in the dried state, and seem to be unusually fragile and very readily damaged and fragmented in purification procedures. Adenosinetriphosphatase activity in the plasma of birds with myeloblastosis, closely associated with the presence of virus and showing a correlation between enzyme activity and particle content, has been observed by Mommaerts *et al.* (1954). Similar enzyme activity has not been found in erythroblastosis plasma, but this difference may be due to the relatively low virus concentration in erythroblastosis (Eckert, Beard and Beard, 1956)

Immunological studies with myeloblastosis virus concentrates by Eckert and associates (1955) have shown that the particles are able to induce the formation of antibodies in the rabbit reacting with the virus, with normal chick tissues, and with the Forssman antigen. The chick tissue antigens and Forssman antigen could not be separated from the virus

particles by sedimentation or electrophoresis, and specific neutralization experiments strongly suggested that these antigens were integral constituent parts of the virus (Beard, Sharp and Eckert, 1955). Although viral immune bodies do not commonly arise during the course of myeloblastosis, typical antibodies in high titre result from the experimental introduction of high immunizing doses in the chick.

The fowl myeloblastosis virus is one of peculiar interest in view of its demonstrable possession of enzyme activity and the incorporation in its structure of components antigenically indistinguishable from constituents of host tissues. Beard, Sharp and Eckert (1955) argued that these data provide a "substantial experimental basis for the concept that the myeloblastosis virus originated initially from the host cell, maintaining through the years a part of its host identity and developing, with time, the properties of autonomous existence". The findings with this agent therefore lend support to the hypothesis that tumour viruses may be modified host proteins in essence, perhaps in some cases arising *de novo* as a result of appropriate physical or chemical stimulation of the cell, capable of self-production and able to produce neoplastic change in the cells of the host without the introduction of any infective agent from external sources. Perhaps relevant to this question is the fact that chicken erythroblastosis and myeloblastosis, though caused by highly infectious viruses and rapidly and easily transmitted by cell-free filtrates, show no evidence of natural spread by either direct or indirect contagion. In this respect they contrast sharply with visceral lymphomatosis which is transmitted to susceptible chicks directly by contact with naturally or artificially infected fowls or indirectly by contact with infected environment (Burmester and Gentry, 1954). There is also some evidence that a form of "vertical" transmission from parent to offspring may occur in avian visceral lymphomatosis, and embryonated eggs and chick tissue even from apparently healthy parents may contain the disease agent (Cottral, Burmester and Waters, 1950). This observation provides an interesting parallel with the findings in certain transmissible mouse leukaemias to be discussed later.

Leukaemia in the Mouse

A very wide range of proliferative disorders arising from haemopoietic tissues has been encountered in the mouse, including "leukaemias" of lymphocytes, granulocytes, monocytes, mast cells and plasma cells Very few of these diseases, however, appear to resemble

in man (Furth, Ferris and Reznikoff, 1935, Kaplan, 1954). Dunham and Stewart (1953), in a valuable survey of transplantable and transmissible animal tumours, listed 37 examples of "lymphomas and leukaemias" known to be established in mice at that time. The majority of these were lymphocytic, 29 being included under the heading "lymphosarcoma or lymphocytic leukaemia, or both, in most instances." The remaining 8 tumours were composed of 4 myeloid leukaemias, 2 reticulum-cell sarcomas, a reticulum-cell leukaemia and a plasma cell leukaemia. While this list is not comprehensive and could now be greatly expanded, the general picture of "leukaemia" in the mouse as a predominantly lymphocytic disease, and of lymphocytic leukaemia and lymphosarcoma as

peculiarly closely related and often almost indistinguishable, certainly remains true as far as transplantable tumours in the mouse are concerned. A considerable proportion of experimental work in this field in the past has involved lymphosarcoma rather than leukaemia, with invasion of blood and bone marrow usually a minor feature (Kirschbaum and Strong, 1939; Kaplan, 1947), and even when blood and bone-marrow involvement has been conspicuous, massive local tumour formation at the site of inoculation, or in lymph nodes, with areas of necrosis and marked histological invasiveness recall the morphology of lymphosarcoma in man rather than that of either acute or chronic lymphatic leukaemia. Nevertheless, "leukaemia" is now generally accepted as an appropriate name for these transplantable mouse tumours, although workers have usually been careful to emphasize the differences between the experimental mouse leukaemias and the disease in man, and to point out that "leukaemic grafts and the animals bearing them are not exact analogues of either the growths or the hosts in spontaneous disease" (Hauschka and Furth, 1957).

Among the contributions to our knowledge of the aetiology of leukaemia derived from studies of the disease in mice are observations on genetic influences and constitutional factors such as age, sex and state of nutrition, the importance of external leukaemogenic agents and internal hormonal mechanisms, and the role of the thymus. Informative reviews of various aspects of this subject include those of Furth (1951), Kirschbaum (1951), Kaplan (1954), Law (1954*a* and *b*), and Hauschka and Furth (1957) A further and most interesting development has been the discovery reported by Gross in a series of publications since 1951 that certain varieties of mouse leukaemia are transmissible by a filtrable agent, presumably a virus, and that virus transmission may occur vertically in a family of mice from one generation to another directly through the embryos (Gross 1954*a*, 1957).

Genetic influences and spontaneous mouse leukaemia

Susceptibility to leukaemia in the mouse is unquestionably strongly dependent upon genetic constitution. Long-continued pedigree inbreeding has produced strains of mice of uniformly high or low leukaemia incidence, corresponding to the uniformity of their inbred genetic make-up In the much studied high-leukaemic strain AKR, for example, the disease develops invariably at about six months to a year, and all AKR mice that escape an earlier death from pneumonia and survive to this age die from leukaemia. Similarly, the C58 strain has a leukaemia incidence of 90 per cent, while even the 10 per cent failing to develop the disease yield, when mated, offspring with the same high leukaemia rate as offspring from leukaemic C58 parents Crossbreeding experiments, using high-leukaemic C58 males and low-leukaemic females of the STOLI strain, and backcrossing the first hybrid generation males with STOLI females, produced families of the second generation having wide differences in leukaemia incidence, ranging from 0 to 43 per cent (MacDowell and Richter, 1935) Such variations are most suggestive of the reassortment of more than one gene influencing susceptibility to leukaemia, since, if only a single gene were involved, the second hybrid generation might be expected to show a bimodal pattern of incidence, according to whether the gene had or had not been transmitted, rather than the continuous variation in fact found (Law, 1954*b*) Moreover, the pattern of leukaemia incidence in the families exhibited the diversity to be expected as a

result of gene redistribution, rather than the uniform moderate incidence which would probably result from breeding with a low-leukaemic strain if the high C58 incidence were originally due to a non-genetic transmissible pathogen, such as a cytoplasmic virus (Law, 1957). This is not, of course, to say that genetic factors are alone responsible for the development of leukaemia in high-incidence strains of mice, but merely that genetic constitution influences susceptibility to the disease. Leukaemia is likely, *in fact, to result* from the combined action of genetic and non-genetic factors. This combination effect is illustrated by the observations of Kirschbaum and Mixer (1947) that strains of mice with low incidence of spontaneous leukaemia might show characteristic patterns of susceptibility to external leukaemogenic agents, the degree of susceptibility or refractorIness to various specific leukaemogens being apparently associated with genetic constitution.

Genetic influences and leukaemia transplantation

The fate of transplanted leukaemic tissue, like that of other normal or neoplastic grafts, is greatly influenced by the antigenicity of the transplanted cell surfaces in relation to the antibody-producing activity of the host. Gorer (1942) first investigated this aspect of leukaemia transplantation in the mouse. Several of the most important cellular antigens appear to be determined by a single gene complex, histocompatibility-2 (H-2), and serological analysis has demonstrated at least eight distinct antigens under the control of this complex Allen (1955) located H-2 on the ninth chromosome, and produced evidence that crossing over might occur within this locus Although H-2 appears to be the most important histocompatibility locus, several others undoubtedly exist, and together they influence the survival of transplanted cells through immunological mechanisms. Probably metabolic and hormonal activities under genetic control, which may differ in donor and host, are also of importance in the success or failure of the graft The increasing complexities of this field of study, the work on active transplantation immunity and its passive transfer, and evidence for the possible existence of new antigens in leukaemic mouse leucocytes, are extensively reviewed by Hauschka and Furth (1957).

Influence of age and sex

Age appears to influence susceptibility to leukaemogenic agents, perhaps as a result of intrinsic changes in haemopoietic tissues or as a consequence of altered hormonal activity. Kaplan (1948) showed that C57BL mice developed lymphoid tumours and leukaemia after whole-body X-radiation much more frequently when less than 6 months old than when over this age. Similarly, DBA/2 mice became leukaemic in response to methylcholanthrene only when treated early in life, although older mice might have their susceptibility increased by removal of the gonads (Kirschbaum, 1957).

The increased incidence of human leukaemia in males as compared with females has already been noted. In mice the reverse is true, especially in certain high-leukaemia strains (Richter and McDowell, 1935), and in AKR mice spontaneous leukaemogenesis may be inhibited by androgens (Murphy, 1944).

Nutritional state

The incidence and rate of growth of tumours in general is decreased by simple calorie restriction (Tannenbaum, 1947) and leukaemogenesis can be similarly inhibited (Saxton,

Boon and Furth, 1944). The mechanism of this action is poorly understood, although dietary deficiency of sulphur-containing amino-acids may be important (White and Mider, 1941), and susceptibility to leukaemia may also be modified by changes in fat intake (Lawrason and Kirschbaum, 1944). The nutritional state must clearly be carefully controlled during experimental studies on leukaemogenesis and its inhibition by various agents, since a misleading impression of specific inhibition might be derived from the use of a drug or hormone which impaired nutrition.

Ionizing radiations in experimental leukaemogenesis

Exposure to X-rays or other ionizing radiations has long been known to be leukaemogenic in mice and other laboratory animals. When leukaemia is induced by radiation in man the disease is usually of the myeloid variety, but in mice lymphoid tumours or lymphatic leukaemias are nearly always produced, and only a few strains develop myeloid leukaemia with any frequency. The subject has been reviewed by Furth and Upton (1953) and by Furth an

action of

exposed

in mice, although there are marked genetic differences in susceptibility in different strains. Other common laboratory animals, rats, guinea-pigs and rabbits, appear to be relatively resistant, to judge from the restricted studies carried out with these species. Repeated exposures are more effectively leukaemogenic than a single larger dose, and the induction rate increases with increase in total dose, but although lymphopoiesis may be depressed by doses as low as 25 r, leukaemia is rarely induced by doses much less than 200 r in sum. Whether the relation between dosage and leukaemia incidence is curvilinear or straight-line at low levels has not apparently yet been established with certainty, although the studies of Kaplan and Brown (1952*a*) and of Finkel (1958) suggest the existence of a minimal effective dose.

The incidence of radiation-induced leukaemia, like that of the spontaneous disease, may be influenced by several agencies to be discussed more fully later. Among these thymectomy and cortisone administration inhibit leukaemogenesis, while adrenalectomy increases susceptibility (Kaplan, 1950; Kaplan, Marder and Brown, 1951)

The mechanism of leukaemia induction by radiation remains uncertain, and much of the work related to this problem has been done on "leukaemias" originating as thymic

in 64 per cent In each case repeated exposures of comparable amount were given for a period of 12 weeks The implications of this observation have been further clarified by the

finding that direct irradiation of the thymus, with the remainder of the body shielded, did not provoke leukaemias of thymic origin, while a protective effect against the leukaemogenic action of whole-body irradiation could be achieved by shielding one hind leg (Kaplan, 1949, 1951; Kaplan and Brown, 1951). Similar inhibition could be brought about by spleen shielding (Lorenz, Congdon and Uphoff, 1953) or, even more remotely, by injecting homologous bone-marrow suspensions intravenously into unshielded totally irradiated mice (Kaplan, Brown and Paull, 1953). A close relation must exist between these inhibitory effects of organ shielding or tissue injections on leukaemogenesis and the widely studied protective action of organ shielding and injection of spleen or marrow homogenates against acute radiation injury and death in mice The intimacy of this relation is emphasized by Cole, Nowell and Ellis (1956), who found that X-irradiated mice, protected from acute radiation death by intraperitoneal inoculation with homogenates of isologous mouse splenic tissue, showed a marked decrease in lymphoma incidence as compared with non-irradiated control mice, whereas, in the experiments of Furth and his associates (1954), unprotected survivors of the same strain of mice (LAF1), after exposure to radiation from a nuclear detonation, showed an increase in both thymic and generalized lymphomas. It seems reasonable to conclude that the spleen homogenate provided protection against both the short-term danger of acute radiation death and the long-term risk of developing leukaemia or lymphoma Most of the studies on the nature of this protective agency have been concerned with the acute risk, but the results of this work are clearly relevant to the problems of radiation leukaemogenesis, and it is pertinent to summarize them here

The weight of evidence at present suggests that protection from radiation death can be achieved by shielding haemopoietic tissue during the period of exposure, or later injecting haemopoietic cells, so that multiplication of the shielded or injected cells can bring about replacement of the blood-forming tissues destroyed by radiation This conclusion is supported by the results of several different experimental approaches.

(*a*) *Lead shielding of the spleen and other organs* Jacobson and his associates first demonstrated in 1949 that lead shielding of the surgically exteriorized spleen of adult mice during exposure to total-body X-irradiation greatly increased the survival rate Subsequent work by this group showed that when mice were exposed to 1,025 r total-body radiation in a single dose, survival of unshielded animals was less than 2 per cent, whereas shielding of the spleen enhanced survival to 76 per cent, and approximately 30 per cent survival followed lead shielding of portions of exteriorized liver or intestine, or the entire head or one hind leg. Survival was not improved by shielding an exteriorized kidney. More detailed examination of these data with regard to rapidity of haematological recovery of survivors, and comparative weights of tissues with different histological components which required shielding to produce an equivalent protective effect, suggested that the important factor was the content of reticulo-endothelial cells and potentially [illegible] Jacobson, 1954).

[illegible] Soon after the efficacy of spleen [illegible] of survival was brought about by transplanting homologous spleens or embryonic tissue and by intraperitoneal or intravenous injection of spleen or embryo cell suspensions during the immediate post-irradiation period, preferably within the first few hours, although some effect could be

obtained with treatment given as late as 2 or 3 days after exposure (Jacobson, 1952). That injection of bone-marrow suspensions could give equal protection was shown by Lorenz and his colleagues, using first homologous marrow in mice and guinea-pigs (Lorenz [illegible]

biosis provides a marked degree of protection against injury when one partner is irradiated (Barnes and Furth, 1943). The effect also becomes manifest if irradiated animals are joined to non-irradiated litter mates within a few hours after exposure. Brecher and Cronkite (1951) obtained 50 per cent survival in rats so treated after exposure to a dose of whole-body X-irradiation which proved uniformly fatal to controls

In general reviews of the field, Jacobson (1954, 1956) discussed the mechanism of the protective action, and concluded that recovery was more probably due to a humoral agent contributed by the shielded or injected tissues than to cellular colonization. In favour of this view was the efficacy of heterologous material which had not been shown to be compatible, and the studies of Cole and his associates (1952, 1953, 1955), who found that homogenized splenic tissue, in which the cells were largely disrupted, was still protective. The active material was shown to reside in the heaviest centrifugal residue and to be destroyed by deoxyribonuclease and trypsin, and was therefore thought to consist of deoxyribonucleoprotein, but the presence of intact cells in the homogenates and in the active centrifugates could not be excluded. Nevertheless, at this stage the humoral hypothesis appeared the more likely

Within a short period, however, clear evidence to the contrary was published from several laboratories Lindsley, Odell and Tausche (1955) irradiated rats having type D blood and injected bone-marrow from rats of the same strain but with type C blood. Donor red cells were identified serologically in the circulation of the host, greatly predominated after several weeks, and persisted for some months The authors did not use fully lethal doses of radiation and could not correlate donor-cell proliferation strictly with survival of the host rats, but their studies favoured graft establishment rather than a humoral factor as of chief importance. Further support for this hypothesis was soon provided. Ford and his colleagues (1956) exposed CBA mice to 950 r of X-rays and then injected them intravenously with cell suspensions from the spleens of infant mice having a conspicuous abnormal chromosome. The marker chromosome showed up prominently in bone-marrow Feulgen-squash preparations. Irradiation survivors were sacrificed at intervals up to 49 days, and in every case the marker was easily identified in the great majority of bone-marrow cells showing well-spread chromosomes. No cells could be found having a full complement of chromosomes, but without the marker. In the same paper the authors reported a second series of experiments in which irradiated mice were injected with a suspension of bone-marrow cells from Wistar rats Surviving mice were found, at 5, 11 and 19 days after injection, to have readily identifiable rat chromosomes in bone-marrow preparations, whereas no certain mouse cells in division were seen Two different methods, one using homologous and the other heterologous material, had therefore established colonization by the donor cells as the mechanism of protection and recovery. Another elegant demonstration of this mechanism was provided by Nowell and his associates (1956), using the histochemical contrast between mouse leucocytes, which give

a negative reaction for alkaline phosphatase, and rat leucocytes, which are rich in this enzyme. Irradiated mice were injected with rat bone marrow an hour after exposure. During the stage of active haemopoietic recovery, beginning about 7 days later, over 80 per cent of the marrow leucocytes were phosphatase-positive, and therefore presumably rat cells. This picture of phosphatase-positive cells predominating in the marrow was still found in surviving animals up to 30 days. By this time, however, deaths were occurring frequently among the mice, presumably from a delayed immunological reaction to the incompatible heterologous rat cells as the immune system of the hosts recovered from irradiation. Data on marrow content after 4 weeks was therefore very limited, but some mice showed evidence of a transition from phosphatase-positive to phosphatase-negative leucocytes in the blood and bone marrow, so that reversion to haemopoiesis by native mouse cells may have been taking place. On the other hand, in a few late survivors, phosphatase-positive cells were still present in blood smears up to 16 months after irradiation. Similar results were reported by van Bekkum and his co-workers, who used, in addition to enzyme histochemistry, a serological method of identifying red cells with specific antisera. In irradiated mice treated with rat marrow the peripheral blood showed, by the eighth day, a falling proportion of erythrocytes reacting with anti-mouse cell serum, and a rising proportion agglutinated by anti-rat cell serum. In some mice the host cells had been entirely replaced by rat erythrocytes after 60 days, and this complete replacement was still encountered as long as 200 days later, but in others a temporary transition was followed by total disappearance of heterologous cells and recovery of normal mouse erythropoiesis Yet another group showed partial replacement, with mixed erythrocyte content, maintained in some cases for 300 days (Vos *et al.*, 1956, Bekkum, Vos and Weyzen, 1956).

The inference to be drawn from these parallel studies is clearly that recovery from acute radiation damage after treatment with homologous or heterologous cell suspensions is dependent upon acceptance and regeneration of the transplanted tissue, and that continued survival is often associated with complete or partial replacement of haemopoietic tissue by cells from the graft. Loss of grafted cells and recovery of the survivor's own haemopoietic activity may sometimes occur after a variable period, but has certainly not yet been shown to be the rule (Bekkum and Cohen, 1957).

In all this experimental work, it is to be noted that organs whose shielding has proved protective and tissues which enhance survival are invariably rich in haemopoietic elements and primitive multipotential cells. Injections of thymus cell suspensions have failed to afford protection from acute radiation death, nor do they promote regeneration of the thymus and lymph nodes after radiation injury, as bone-marrow and spleen cells do (Brown *et al.*, 1955; Cole and Ellis, 1955). Cole, Nowell and Ellis (1956) therefore argued that induced thymic regeneration in irradiated mice protected by bone-marrow injection is the result of an indirect mechanism, secondary to the establishment of the marrow graft. A similar indirect mechanism might also inhibit the later production of leukaemias and thymic lymphomas Certainly the efficacy of partial body shielding and tissue transplantation in preventing radiation leukaemogenesis is difficult to explain on the basis of simple repopulation of damaged areas, since the majority of tumours arise in the thymus rather than in the spleen or bone marrow, and there is no evidence of thymic repopulation by cells from the graft.

Influence of chemical carcinogens and hormones on leukaemogenesis

It has long been established that carcinogenic hydrocarbons will induce leukaemia in mice, one of the most potent being 9,10-dimethyl-1,2-benzanthracene (Law, 1941). This substance is leukaemogenic upon percutaneous application or subcutaneous injection, although there is evidence, at least in Street mice, that the site of injection may be important in influencing both the development and the manifestations of leukaemia (Rask-Nielsen, 1949*b*), perhaps because of varying rates of absorption from different sites. Other hydrocarbons such as 3,4-benzpyrene, 1,2,5,6-dibenzanthracene and 20-methylcholanthrene are less effective, producing thymic lymphomas when directly injected into the thymus, but not when applied more remotely in Street mice (Rask-Nielsen, 1949*a*, 1950). Genetic and strain differences in susceptibility to carcinogens are conspicuous, however, and leukaemia induction by skin-painting with benzpyrene has been demonstrated in dilute brown mice (Morton and Mider, 1941), with dibenzanthracene and with methylcholanthrene in strain F mice (Kirschbaum and Strong, 1942), in C3H mice (Morton and Mider, 1941) and in several other strains. Kirschbaum (1957), in illustration of the remarkable genetic differences in susceptibility, instanced the failure of C58 mice, with a high incidence of spontaneous leukaemia, to respond to the leukaemogenic action of methylcholanthrene, whereas the low-leukaemia strain DBA/2 is very susceptible. The strain differences are not necessarily related to general levels of tissue resistance but to varying sensitivity of target organs. Thus Strong A mice treated with methylcholanthrene produce lung and mammary neoplasms but not leukaemia, while DBA/2 mice similarly treated readily produce leukaemia and mammary tumours but not lung cancer. Leukaemia and lymphoma induction by chemical carcinogens, as by radiation, may be profoundly influenced by hormonal factors and thymic activity.

Lacassagne (1937) first drew attention to the leukaemogenic potency of oestrogens in mice, and Gardner, Dougherty and Williams (1944) were able to increase the incidence of the disease in low-leukaemia strains from 2 per cent to 25 per cent by oestrogen administration. The mode of action of oestrogens is uncertain, but it is clear that, once again, susceptibility is greatly dependent upon genetic constitution A greater incidence of leukaemia and lymphoma follows short repeated treatment with oestrogen than prolonged courses, although there may be a considerable latent period, up to 12 months, before the disease becomes manifest (Furth, 1946; Burrows and Horning, 1947) The type of leukaemia produced is usually thymic in origin, and the genesis of similar leukaemias induced by X-rays or chemical carcinogens may be potentiated by exogenous oestrogens (Kirschbaum, Shapiro and Mixer, 1949, 1953).

Androgenic hormone contrasts sharply with the oestrogens, for exogenous testosterone administration will suppress the genesis of thymic lymphoma and leukaemia, whether [illegible] 1944), by X-rays (Gardner, [illegible] (Kirschbaum and Liebelt, [illegible] disease in high-leukaemia strains (Murphy, 1944)

With regard to the effects of endogenous sex hormones on leukaemia incidence in mice, there is evidence that they exert some influence, either supportive or inhibitory, in both spontaneous and induced leukaemogenesis The experimental studies have been extensively reviewed by Dougherty (1952) and by Kaplan, Nagareda and Brown (1954).

Miller and Pybus (1942) found a marked increase in the incidence of lymphoid tumours after gonadectomy in both male and female mice of the Edinburgh strain, and Buffet and Furth found ovariectomy to enhance leukaemia incidence in LAF1 mice (Hauschka and Furth, 1957), but the more general experience has been that removal of the ovaries is either inhibitory or without effect. Orchidectomy, on the other hand, has led to a greater incidence and shorter latent period before onset of the disease in all strains of mice studied (McEndy, Boon and Furth, 1944, Murphy, 1944, Law, 1947; Arentsen and Hogreffe, 1951). The high incidence of leukaemia in castrated male mice can be reduced by treatment with testosterone propionate

Inhibition of leukaemogenesis can also be brought about by adrenocorticotrophic hormone and the cortisone group of steroids (Kaplan, Marder and Brown, 1951; Woolley and Peters, 1953), but desoxycorticosterone acetate does not affect the incidence The removal of endogenous cortisone activity by adrenalectomy is followed by an increase in lymphoma incidence (Law, 1947)

Other hormones have not been shown to affect leukaemogenesis in mice, although hypophysectomy may inhibit the induction of certain other tumours (Ferguson and Visscher, 1953) and continued administration of pituitary growth hormone has been reported to induce lymphosarcoma in rats (Moon and Li, 1950). Hypophysectomized irradiated C57BL mice, in the experience of Nagareda and Kaplan (1954), developed lymphoid tumours without inhibition

Kaplan (1954) has emphasized the effects of these endocrine agents on the mouse thymus, pointing out that all the hormones inhibiting lymphoma development also produce involution of the thymus, while procedures such as orchidectomy and adrenalectomy which enhance tumour incidence also cause thymic hypertrophy The action of oestrogens is alone contrary to this general rule, for here increased tumour induction and thymic involution go together

The role of the thymus in mouse leukaemia

The frequent origin of mouse lymphosarcomas and lymphatic leukaemias in the thymus has been continually emphasized during the previous discussion, although it is true that not all strains of mice and not all varieties of mouse leukaemia show this feature Nevertheless, the thymus has occupied a more prominent place than any other organ in experimental studies of leukaemogenesis.

McEndy, Boon and Furth (1944) first demonstrated that thymectomy was followed by a remarkable fall in the incidence of leukaemia of thymic origin in the high-leukaemia AK strain of mice, and Law and Miller (1950*a*) later showed that the operation was also effective in reducing the incidence of spontaneous leukaemias originating elsewhere. Thymectomy similarly inhibited the induction of leukaemia by X-rays in C57BL mice (Kaplan, 1950) and by methylcholanthrene in DBA/2 mice (Law and Miller, 1950*b*). Studies of leukaemia development in thymectomized animals subsequently provided with implanted thymic tissue have given most interesting results Law and Miller (1950*b*) thymectomized F1 hybrids from a high- and low-leukaemia strain cross. When these hybrids were grafted with thymus from the high-leukaemia parent stock they developed a high incidence of spontaneous leukaemia, but when grafted with thymus from the low-leukaemia parent stock they showed a low incidence. Transplantation experiments proved

that the proliferating leukaemic cells did not originate from the graft, but from the host,

X-rays and then implanted thymic grafts. Leukaemic transformation occurred in the cells of the non-irradiated graft. Similar studies by Law and Potter (1958) enabled them to confirm that such a transformation could take place, and they demonstrated that the post-irradiation leukaemogenic influence persisted for at least 28 days.

These experiments suggest that the thymus may act either by providing a genetically determined leukaemogenic stimulus to other lymphocytic tissues or by responding to an indirect induction stimulus originating elsewhere in response to radiation or chemical carcinogens. Kaplan (1954) has put forward a unifying hypothesis which attempts to explain the mechanism of lymphosarcoma and leukaemia induction, however initiated, as dependent upon the intensity of a postulated proliferative stimulus on the thymus and the capacity of this organ to respond. When the lymphatic system and thymus are injured

shielding or bone-marrow transplantation is to allow thymic regeneration to proceed more rapidly to meet the stimulus, or perhaps to reduce the intensity of the stimulus, and so prevent the development of lymphoid tumours. Hormones which inhibit leukaemogenesis do so, in this theory, by reducing the general demand for thymic activity, a reduction which leads to thymic involution in normal animals, and to a depression of the thymic proliferative stimulus in those exposed earlier to leukaemogens. The essential leukaemogenic factor would therefore be an endogenous growth stimulus acting upon a poorly responsive thymus. Although the presence of thymic tissue appears to be essential for the development of lymphoma in many strains of mice, the complexity of the intermediate mechanisms in tumour induction is so great that hypotheses like that of Kaplan have to postulate the existence of multiple factors to explain the experimental observations, and no explanation carrying full conviction is yet available

Transmission of leukaemia by cell fragments

The concept of transduction, the transfer of subcellular particles from one cell to another with a consequent character change in the recipient cell, is well established in bacterial

1953; Lederberg, 1956). The phenomenon has not been shown to occur in mammalian cells, although some such mechanism might explain the alteration in histocompatability patterns of transplantable mouse lymphomas after passage through F1 hybrids between susceptible and resistant strains (Hauschka and Furth, 1957). If transfer of subcellular transforming agents does take place in mammalian tissues, the question arises whether the malignant characteristics of tumour cells might not be transmitted to normal cells by this means. Some relevant early studies in this field were carried out by Stasney, Cantarow and Paschkis (1950, 1952), who attempted to transmit hepatomas and lymphosarcomas

in the rat by isolated subcellular fractions of the neoplastic cells, and succeeded in inducing lymphosarcoma formation, usually at the site of injection, after subcutaneous introduction of chromatin material and, less frequently, mitochondria from malignant lymphocytes. The authors could not absolutely exclude the presence of some intact tumour cells in the material injected, although none were seen in sample preparations. The existence of even very small numbers of viable cells in the preparations would certainly cast doubt on the conclusions, since tumour transfer, with a considerable proportion of takes, can be achieved with single cells, at least in the mouse. The Murphy rat lymphosarcoma, with which Stasney and his associates worked, is, however, apparently less easily transferred, an inoculum of some 3,000 neoplastic lymphocytes being needed to produce effects comparable to those of the chromatin fractions (Paschkis, Cantarow and Stasney, 1955). The authors claimed that such a number of cells could not possibly be present in their material without detection, and the easy recognition of many intact tumour cells when 3,000 neoplastic lymphocytes were experimentally added to a sample chromatin suspension strongly supported their claim This work can hardly be said to provide unequivocal proof of transduction as a mechanism of leukaemogenesis, but it does bring the weight of considerable experimental evidence in favour of the concept that chromatin fragments from malignant cells may be taken up by normal leucocytes and incorporated in their nuclei, presumably during the processes of mitosis and DNA synthesis, so conferring neoplastic characteristics upon initially normal cells

If the transduction mechanism is the true explanation of Stasney's results, the induced lymphosarcoma cells might be expected to have, in general, the genetic constitution of the host rather than that of the original tumour from which the chromatin fraction had been derived Klein (1952) made chromatin extracts from DBA/2 lymphoma and 6C3HED lymphosarcoma, and injected one or other of these extracts intraperitoneally into F1 hybrids between DBA and C3H mice The tumours produced could be successfully transplanted to the susceptible parent strain, whereas grafts of F1 hybrid tissue normally take only in hosts carrying the iso-antigens of both parent strains. This evidence suggests that the induced tumours had the constitution of the original tumour cells and not that of the F1 host, and arose from proliferating implanted viable cells rather than by transduction. However, as Hauschka and Furth have pointed out, there are exceptions to the general rule about compatibility of F1 grafts, leukaemias arising spontaneously in F1 hybrids being occasionally transplantable to one parental strain (Schweitzer and Furth, 1939). Moreover, introduction of malignant chromatin material into F1 leucocytes might be expected to produce a chromosomal imbalance which could lead to an alteration of the histocompatibility antigens The resulting cell might then transcend the normal immunogenetic rules of transplantation. The question of chromatin transmission of leukaemia by transduction therefore still remains open.

Transmission of leukaemia in mice by viruses

In 1951 Gross first reported the successful induction of leukaemia in mice of the low-leukaemia C3H strain after inoculation in infancy with cell-free material from AK leukaemic tissue or normal AK embryo extracts Subsequent studies by Gross (1952*a* and *b*, 1953*a*, *b*, *c* and *d*, 1954*a* and *b*, 1955*a*, *b*, *c* and *d*, 1956*a* and *b*, 1957) have greatly extended

his original observations. The most important findings from this long series of experiments may be summarized as follows.

Extracts prepared from AK or C58 leukaemic mice, passed through Berkefeld N or Selas 02 or 03 filters and inoculated into newborn mice of certain susceptible low-leukaemic strains, induced leukaemia in about 25 per cent of the test animals after a latent period of several months. Heating to 68°C. for 30 minutes inactivated the filtrates and reduced the incidence of leukaemia in inoculated mice to about 2 per cent, a figure little above the normal spontaneous rate in the strains concerned.

Leukaemia induced in C3H mice by filtered AK extracts could be transplanted to adult

In sharp contrast to this process, leukaemias of AK origin transplanted to C3H mice by cellular implantation led to generalized leukaemia within 2 to 4 weeks, this leukaemic tissue could not be grafted to adult C3H mice but only to mice of the donor AK strain, and was therefore probably derived wholly from the transplanted AK cells

The cell-free leukaemogenic agent was successfully passaged through six consecutive hosts, and during this procedure some enhancement of potency appeared to occur, in that the period of latency decreased after the third passage

Vertical transmission of the infective agent from one generation to the next through the embryos was demonstrated in several ways. Extracts prepared from normal embryos of high leukaemia strains, such as AK and C58, were found to contain the agent Cell-free filtered preparations from these embryonic tissues could induce leukaemia when injected into susceptible newborn mice When C3H mice, inoculated in infancy but not yet frankly leukaemic, were mated, their offspring showed a relatively high incidence of the disease in adult life

Filtration of the leukaemic tissue extracts through Gradocol membranes, ultracentrifugal sedimentation studies, and electron microscopy indicated that the cell-free extracts contained particles having a diameter between 30 and 70 $m\mu$.

Certain complications have tended to obscure the clear-cut picture of viral transmission of leukaemia. In the first place cell-free transmission was successful in the laboratory only when newborn mice, preferably less than 6 hours old, were used for inoculation. When animals over 12 to 16 hours old were used, results were irregular and unconvincing Moreover, attempts by other workers to confirm the earlier observations of Gross met with only partial success, and it soon became obvious that genetic constitution was of major importance, differences between sublines of the same strain being enough to alter susceptibility very greatly. Another difficulty in interpreting the results of inoculation of cell-free leukaemic material has arisen from the ability of such inoculations sometimes to provoke the development of parotid tumours or subcutaneous sarcomas These two problems of genetic susceptibility and alternative tumour formation show some interrelationship. Law, Dunn and Boyle (1955) inoculated newborn C3H mice of two different sublines with filtered extracts of leukaemic tissue from AK or C58 mice. Very few leukaemias appeared, but parotid tumours or subcutaneous sarcomas developed. Woolley and Small (1956), using Bittner subline C3H mice, obtained positive results of leukaemia induction in mice without the milk factor, but failed to produce leukaemia in mice possessing the milk factor.

Stewart (1953, 1955*a* and *b*) attempted to repeat the experiments of Gross, but was unable to produce an increased incidence of leukaemia in newborn C3HF mice by injecting cell-free AKR leukaemia material. When the filtrates were injected into F1 hybrids (C3HF × AKR), however, an increase of leukaemia incidence from 23 to 60 per cent took place, accompanied by a shortening of the latent period before development of the disease. These hybrid leukaemias could not be transplanted to either of the parental stocks, but only in other hybrids of C3HF and AKR, indicating that the leukaemias arose from hybrid cells. In Stewart's experiments cell-free filtrates from AKR leukaemia tissue induced salivary gland carcinomas, while similar material from a C3H subline leukaemia produced parotid tumours in some animals and subcutaneous or suprarenal tumours in others. Gross (1957), further emphasizing these genetic differences, pointed out that while some sublines of C3H appeared to respond to the filtered extracts by producing any of the three neoplasms, leukaemia, parotid tumours, or sarcomas, C57 Brown mice readily developed leukaemia, but very rarely any other tumour.

The three kinds of neoplasm induced by cell-free extracts of leukaemic tissue in C3H mice are morphologically quite distinct Gross has described them in detail. The leukaemias, in his experience, were typical generalized lymphatic forms of the disease, usually with blood and bone-marrow involvement. The parotid tumours were usually bilateral, initially adeno-carcinomatous in type, but later tending to a sarcomatous histological appearance. The subcutaneous sarcomas were hard, white, invasive tumours, microscopically fibrosarcomas, fibromyxosarcomas or giant-cell tumours. Although the induced leukaemias were easily transplantable to adult C3H mice, the other two tumours could be transferred only occasionally and with difficulty. Extracts from the induced leukaemic tissues, rendered cell-free by centrifugation or filtration, and inoculated into newborn C3H mice, led to the development of leukaemia in some, parotid tumours in others and subcutaneous sarcomas in others. Similar experiments with cell-free extracts of the parotid tumours and the sarcomas showed that material from these sources could also induce any of the three neoplasms in inoculated C3H mice Whether the responsible factor is a single agent or a mixture of three separate oncogenic agents has not yet been determined, but there is some evidence (Gross, 1956*b*) that treatment with ethyl ether may destroy the leukaemogenic potency of extracts while leaving the capacity to induce parotid tumours or sarcomas unimpaired, and this observation might suggest that three agents exist, the leukaemic one being more sensitive than the others If this were the case, extracts of parotid tumours induced by ethyl ether treated material should in turn be incapable of inducing leukaemia when inoculated into newborn C3H mice.

A further complexity in this field arises from the work of Graffi and his colleagues (Graffi *et al.*, 1954, Graffi and Schmidt, 1954, Schmidt, 1955; Fey, Graffi and Bielka, 1955; Bielka, Graffi and Fey, 1955, Graffi *et al.*, 1956). They inoculated newborn mice with filtered extracts from various solid tumours, including sarcoma 37, sarcoma 594, an ascitic sarcoma, and Ehrlich carcinoma. Lymphocytic leukaemia developed in nearly 50 per cent of the injected mice, the Ehrlich tumour giving the highest percentage. Apart from

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extract administered These studies are open to some criticism. The host animals used

were mice of the Agnes Bluhm line, which is probably not homozygous, and the leukaemias obtained were unusual in that they could not be transplanted. Again, the comparisons between test and control mice were made only at 9 months, and differences might have been less conspicuous if a wider age range had been considered, but these objections are probably not of great importance.

In all this work on leukaemia transmission by filterable agents the type of leukaemia involved has been lymphocytic. Friend (1957) isolated a similar agent from a Swiss mouse; it induced granulocytic leukaemia when transmitted to adult mice of the same strain. Woolley (1957) confirmed these findings, and by inoculating newborn mice was able to transmit the disease to the C3H strain, where the resulting leukaemia continued to be granulocytic in cytology, with positive peroxidase reaction in the cell granules.

Clearly, much experimental work remains to be done with these virus-like agents, in particular to clarify their relationships with genetic factors, physical and chemical leukaemogenesis, hormonal and thymic intermediary mechanisms, protective grafting of haemopoietic tissue, and transduction.

Transmission Experiments with Human Leukaemic Material

Deliberate attempts to transmit leukaemia from man to man have been few and invariably unsuccessful. Thiersch (1945) injected blood or cellular emulsions from the spleen or lymph nodes of leukaemic patients into volunteer recipients, already suffering from malignant disease. The subcutaneous or intravenous routes were used for these inoculations. During an observation period of 24 months the recipients showed no sign of developing leukaemia. In further experiments, Thiersch (1946) used sternal bone marrow from untreated cases of acute leukaemia for the transmission attempts, the material being aspirated from the donor and immediately injected into the sternal marrow of the recipient without the use of anticoagulants. No evidence could be detected clinically or haematologically that the implantations had any effect whatever on the recipients, some of whom were still alive at the end of a period of observation between 21 and 35 months, while those who died during this time did so from the spread of their original malignant tumour.

Similarly negative results were obtained by Bierman and his associates (1950), who set up crosstransfusions between leukaemic and non-leukaemic carcinomatous patients, in the course of which 6 to 10 litres of blood were exchanged hourly. At the end of these

subjects, do not necessarily establish that leukaemia in man differs essentially from the transmissible leukaemias of animals, since histocompatibility of grafts calls for close genetic and immunological relationships between donor and recipient, such as could be encountered in man only between identical twins. Transplantation of leukaemic cells in man could not therefore be expected under the experimental conditions employed. The question still remains whether a subcellular agent or virus might be expected to prove leukaemogenic, if it existed in human leukaemic tissues. Here again, the experiments throw no light on this possibility, for the viral transmission of rodent leukaemias is characterized by a latent period far longer in relation to the life-span than the periods of

observation in the studies of Thiersch and Bierman, is successful predominantly in new-born animals, whereas the human recipients were elderly, and is governed to a marked extent by genetic relations.

Material from human leukaemia has often been introduced into laboratory animals in attempts to show the infective nature of the disease. In general these experiments have been unproductive, but pathological changes in the inoculated animals have been reported from a few centres. These changes have not usually been of a leukaemic nature, are peculiarly difficult to interpret, and have not readily been reproduced by other workers. Torrioli and his co-workers, in a series of reports since 1941, have described the development of a *"mesenchymal reticuloendotheliosis" in chick embryos inoculated with human leukaemic leucocytes from each cytological form of the disease (Torrioli and Torrioli, 1951).* The average morbidity among injected embryos was 63 per cent, and most of these embryos died before hatching. Malformations, including failure of visceral and cephalic development, were common, and chicks surviving long enough to hatch showed anaemia, bone-marrow aplasia, hepatic atrophy and degeneration and diffuse reticulo-endothelial hyperplasia. Successful passage from egg to egg could be achieved by the use of heart blood from inoculated embryos. Pulped tissue from affected chicks readily transmitted the disease, with progressive mortality up to 77 per cent, on successive passages. When tissue pulp was spread on the allantoic membrane cellular reduplication with thickening and stratification of the ectoderm took place and inclusion bodies were seen in the ectodermal cells. The authors inferred from these results that human leukaemia is due to a transmissible virus

Magrassi and his associates have reported since 1946 a further series of experiments which they interpret as evidencing the viral aetiology of human leukaemia (Magrassi *et al.*, 1951; Magrassi, 1955). Material from human cases of each cytological variety was injected into guinea-pigs; plasma, either enriched with leucocytes or with most of the cells removed, was given intraperitoneally, serum intracerebrally, and bone marrow into the marrow cavity. After a variable incubation period, sometimes only a few days, a characteristic pathological reaction occurred in nearly all animals inoculated during the winter months. *During the summer the guinea-pigs appeared to be resistant and did not show symptoms* of disease if inoculated between May and September. The reaction, when it took place, included severe anaemia, loss of hair, skin atrophy, degeneration of glandular parenchyma and proliferation of histiocytes in spleen and lymph nodes. The disease was serially transmissible by splenic and hepatic cell suspensions during the winter months, and passage in this way was easily repeated.

In an extensive series of reports, chiefly in the Spanish and French literature since 1951, Mas y Magro described the transmission of human leukaemia to guinea-pigs by subcutaneous injection, or inoculation of the scarified skin, with fresh or dried blood from cases of the disease. Acute leukaemia, chronic myeloid and chronic lymphatic leukaemia each induced a comparable form of the disease in inoculated guinea-pigs (Mas y Magro, 1952, 1954*a* and *b*). The work has not been independently confirmed, and conflicts with the results of guinea-pig experiments carried out by Magrassi.

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experiments to exclude immediate toxic reactions to the injection of foreign tissues or proteins, and inoculation of control animals with non-leukaemic human cells or plasma gave negative results. Nevertheless, no valid assessment of the results can be made until they have been shown to be reproducible in other hands.

Work in this field has also been carried out in Russia in recent years, but although a few reports have been made available in English translation it is not easy to gather precise information on the experimental methods or the results and their interpretation. Bergol'ts (1957) injected Seitz filtered cell-free saline extracts of lymph nodes from human leukaemic patients into the spleen of adult mice, and reported the development of leukaemia-like changes after 1 to 2 months. Whether the haematological changes observed were leukaemic rather than leukaemoid was difficult to establish, but in one case the induced disease was successfully transplanted to other mice. Mitochondrial cell fractions and nuclear extracts were also found to be actively leukaemogenic. Experimental transmission of leukaemia from man to animals has also been described by Khokhlova (1958), who used benzol extracts from organs of leukaemic patients.

An acceleration of leukaemia development in AKR mice after inoculation with cell-free brain filtrates from human patients who had died of leukaemia has been reported by Schwartz *et al.* (1957). In these experiments, 71 of 326 inoculated animals developed leukaemia before the age of 22 weeks, but the spontaneous incidence of the disease in AKR mice is so high that no conclusion can be drawn until the studies have been repeated in mice of a low-leukaemia strain. In this regard it is of interest to note that cell-free filtrates of leukaemic AKR mouse brain similarly accelerated the induction of leukaemia in mice of the same strain, and also induced the disease in Swiss mice in approximately 50 per cent of inoculated animals, although the spontaneous incidence of leukaemia in Swiss mice of the strain used is not more than 1 per cent (Schwartz, Schoolman and Szento, 1956; Schoolman *et al.*, 1957)

Leucocyte Elimination Studies

The early studies of Minot and Isaacs (1925) showed that transfused leucocytes rapidly disappeared from the peripheral circulation in man, and Isaacs and Danielian (1927) suggested that changes in leucocyte levels might be caused by disturbances of the elimination mechanism. This conception came again into prominence as a result of the cross-circulation studies of Bierman and his associates (Bierman *et al.*, 1950, 1951), who postulated that some leukaemic individuals with high leucocyte counts had an impaired removal mechanism, and that the leucocythaemia might be due as much to lack of normal removal of cells as to excessive production and release. The pulmonary capillary bed was shown to be the chief site of leucocyte removal in these experiments, and was apparently able to eliminate from the blood billions of white cells in a single circulation (Lanman, Bierman and Byron, 1950) Leukaemic leucocytes of any kind were rapidly removed from the circulation of normal recipients, whereas leukaemic granulocytes were only slowly eliminated from the blood of a patient with subleukaemic lymphocytic leukaemia. The capacity of pulmonary capillary endothelium to remove leucocytes has been confirmed in the dog by Ambrus and his associates, who demonstrated that other capillary beds may also filter off leucocytes, and that continued perfusion would eventually satiate the filtration mechanism

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Cell-free materials obtained by filtration or high-speed centrifugation do not seem to have been used in any of these man-to-animal transmission attempts, and the support they lend to the viral hypothesis is indirect. Care has been taken in at least some of these

panied by a normal amount of lymphokentric acid which allowed partial maturation to occur. In acute myeloid leukaemia there was a lack of lymphokentric acid, and the early myeloid cells produced under the influence of myelokentric acid, present in normal or increased amounts, could not mature. Similar pictures were drawn for chronic and acute lymphatic leukaemia, with lymphokentric acid playing a lymphopoiesis-stimulating role and myelokentric acid a lymphocyte-maturing role. The urinary findings in monocytic leukaemia showed an excess of both acids and their function in the disease was open to question.

Attempts to treat leukaemic patients with the appropriate missing acid—for example, acute lymphoblastic cases with myelokentric acid—produced some partial remissions (Miller, Harbut and Jones, 1947), but the evidence at present available in support of the general hypothesis is unconvincing.

The effects of hepatic and splenic extracts from human lymphomata on the lymphoid

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Metcalfe (1956*a* and *b*) reported the detection of a lymphocytosis-stimulating factor in the plasma of patients with chronic lymphocytic leukaemia. Intracerebral inoculation of plasma into 1-day-old mice produced a lymphocytosis when chronic lymphatic leukaemia plasma was used, but no such effect followed the injection of plasma from other forms of leukaemia. The factor was heat-labile but withstood freeze-drying. Its action could be inhibited by cortisone or oestrogens. Extracts from human and mouse thymus and thyroid glands contained the factor, which was present in increased amounts in subjects with chronic lymphocytic leukaemia. The chemical nature of the factor and its possible relationship to the substances described by Miller remains uncertain.

None of these chemical agencies can be regarded as actively leukaemogenic or of primary aetiological importance, but their further study may provide information on the poorly understood mechanisms of physiological control of haemopoiesis and maturation and release of blood cells.

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and allow peripheral leucocytosis to develop (Ambrus *et al*, 1954*a*, *b* and *c*). That cells removed from the blood by capillary networks are not destroyed during the process, but join the non-circulating leucocyte pool, has been made clear by several experiments. Brecher, Wilbur and Cronkite (1953) showed that such sequestrated leucocytes were still available to migrate to sites of infection, while Ambrus *et al.* (1955) reported that adrenaline injections caused atabrine-labelled leucocytes, which had earlier been removed from the blood by capillary filtration, to reappear in the circulation. Osgood (1954) estimated that circulating leucocytes make up less than 1 per cent of the total number of viable leucocytes in the body, the remainder being held in a large reserve "pool". The location of this pool remains uncertain, although many leucocytes may be held in the bone marrow (Craddock, Perry and Lawrence, 1956), and perhaps also in sluggish vascular channels (Hollingsworth and Finch, 1957*b*). Hollingsworth and Finch (1957*a*) have suggested that the extent of saturation of the reserve leucocyte pool may be the factor of chief importance in determining the length of survival within the circulation of transfused leucocytes, and their experiments with leukopenic and leucocyte-saturated rats support this hypothesis. Transfused leucocytes, whether leukaemic or not, were rapidly removed by animals with depleted white cell reserves, whereas they remained much longer in the circulation of animals whose leucocyte pool had been saturated. In either case the cells remained viable after eventual clearance from the circulating blood, and a high percentage of normal rats transfused with leukaemic leucocytes subsequently developed leukaemia.

The delayed clearance of transfused leucocytes by leukaemic patients, observed by Bierman, may therefore have resulted from saturation of the leucocyte pool, and should be regarded as a consequence of the leukaemic proliferation rather than as in any way a cause of the basic disease process. Nevertheless, secondary alterations in the elimination and sequestration mechanism may certainly influence the level of the peripheral white cell count and be of pathogenic rather than aetiological interest

Chemical Factors and Myeloid-Lymphoid Imbalance

Changes in the haemopoietic and lymphoid systems of guinea-pigs injected with extracts of the urine of leukaemic patients led Miller and his associates to suggest that two substances, myelokentric and lymphokentric acids, were of fundamental importance in maintaining a normal balance of leucocyte proliferation and maturation, and might play a large part in the pathogenesis of leukaemias (Miller, Wearn and Heinle, 1939; Miller and Hause, 1940; Jones, Miller and Hause, 1940; Miller and Turner, 1943).

Myelokentric acid was described as a non-carbinol acid found in the urine of patients with acute or chronic myeloid leukaemia, chronic lymphoid leukaemia and monocytic leukaemia. Lymphokentric acid is an hydroxyacid occurring in the urine in acute or chronic lymphoid leukaemia, lymphosarcoma, chronic myeloid leukaemia and monocytic leukaemia. Both acids have also been found in liver lipids and in the urine in Hodgkin's disease, and are believed to exist in conjugated form in the serum of leukaemic patients (Foster and Miller, 1950). Myelokentric acid was said to cause myelopoiesis without maturation, while lymphokentric acid inhibits proliferation of myeloid cells and allows them to divide. Miller and his co-workers claimed that an excess of myelokentric acid existed in chronic myeloid leukaemia, thus causing increased myelopiesis, but was accom-

panied by a normal amount of lymphokentric acid which allowed partial maturation to occur. In acute myeloid leukaemia there was a lack of lymphokentric acid, and the early myeloid cells produced under the influence of myelokentric acid, present in normal or increased amounts, could not mature. Similar pictures were drawn for chronic and acute lymphatic leukaemia, with lymphokentric acid playing a lymphopoiesis-stimulating role and myelokentric acid a lymphocyte-maturing role. The urinary findings in monocytic leukaemia showed an excess of both acids and their function in the disease was open to question.

Attempts to treat leukaemic patients with the appropriate missing acid—for example, acute lymphoblastic cases with myelokentric acid—produced some partial remissions (Miller, Harbut and Jones, 1947), but the evidence at present available in support of the general hypothesis is unconvincing.

The effects of hepatic and splenic extracts from human lymphomata on the lymphoid system of guinea-pigs have been studied by Storer and Lushbaugh (1949), who found that lymphoid proliferation and infiltrations developed; but mineral oils and other substances produced similar effects, and the action of tissue extracts was thought to be non-specific.

Metcalfe (1956*a* and *b*) reported the detection of a lymphocytosis-stimulating factor in the plasma of patients with chronic lymphocytic leukaemia. Intracerebral inoculation of plasma into 1-day-old mice produced a lymphocytosis when chronic lymphatic leukaemia plasma was used, but no such effect followed the injection of plasma from other forms of leukaemia. The factor was heat-labile but withstood freeze-drying Its action could be inhibited by cortisone or oestrogens. Extracts from human and mouse thymus and thyroid glands contained the factor, which was present in increased amounts in subjects with chronic lymphocytic leukaemia The chemical nature of the factor and its possible relationship to the substances described by Miller remains uncertain

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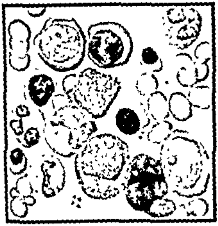
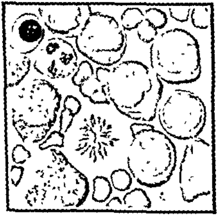
PLATE II

PANOPTIC CYTOLOGY OF NORMAL BONE MARROW AND LEUKAEMIC BLOOD (MAY-GRÜNWALD-GIEMSA STAIN)



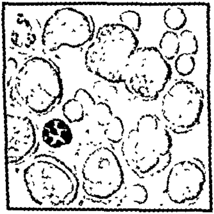
1. Normal bone marrow

4. Acute monocytic leukaemia

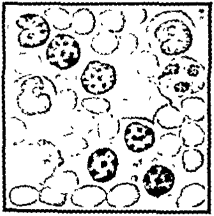


2. Acute myeloblastic leukaemia

5. Chronic granulocytic leukaemia



3. Acute lymphoblastic leukaemia



6. Chronic lymphocytic leukaemia

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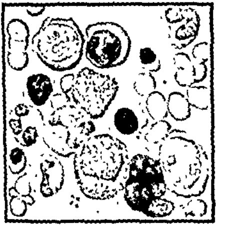
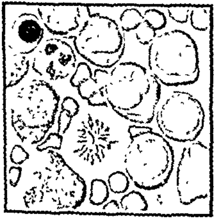
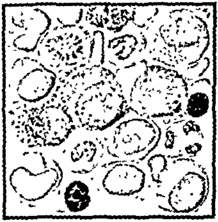


1. Normal bone marrow

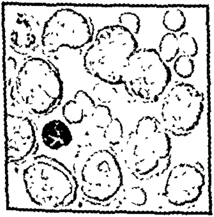
4. Acute monocytic leukaemia



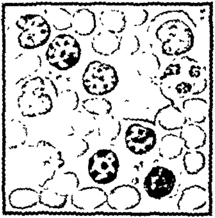
2. Acute myeloblastic leukaemia



5. Chronic granulocytic leukaemia



3. Acute lymphoblastic leukaemia



6. Chronic lymphocytic leukaemia

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PLATE III

ACUTE LEUKAEMIA—MAY-GRÜNWALD-GIEMSA STAIN (M.G.G.) (A)

1. Myeloblasts and a reticulum cell from acute myeloblastic leukaemia, showing the common pattern of uniform nuclear structure with one to four nucleoli and a moderately wide cytoplasmic rim (× 1000)

2. Myeloblasts and a promyelocyte from acute myeloblastic leukaemia An occasional cell shows nuclear folding of the paramyeloblastic kind. (× 1000)

3. A group of less uniform myeloblasts from acute myeloblastic leukaemia, showing variation in cell outline and in nuclear-cytoplasmic ratio (× 1000)

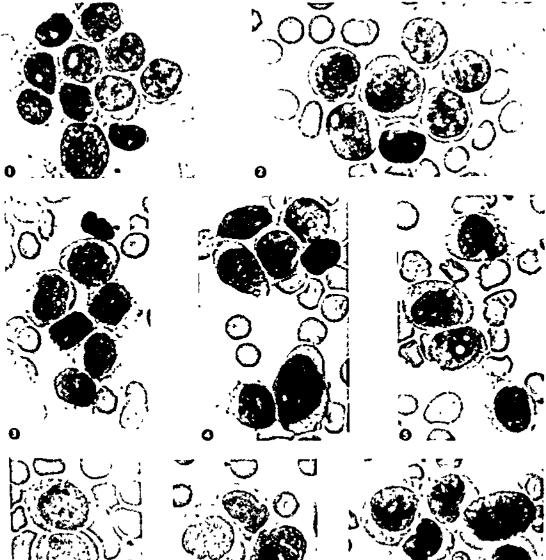
4. Variation in cell size, cytoplasmic irregularities and vacuolation in leukaemic myeloblasts (× 1000)

5. Primitive cells from acute myelomonocytic leukaemia (× 1000)

6, 7 and **8.** Various monocyte precursors from cases of acute monocytic leukaemia (× 1000)

9, 10 and **11.** Auer rods in leukaemic myeloblasts (**9** and **10**) and promonocytes (**11**) (× 1000)

PLATE III



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6, 7 and **8.** Various monocyte precursors from cases of acute monocytic leukaemia (× 1000)

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PLATE IV

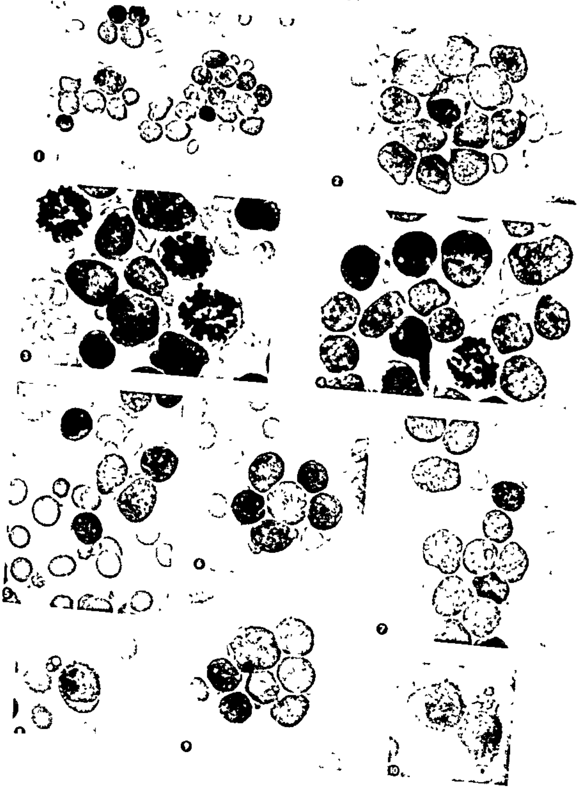


PLATE IV

ACUTE LEUKAEMIA M.G.G. STAIN (B)

1. Typical group of cells from acute lymphoblastic leukaemia, showing high nuclear-cytoplasmic ratio (× 600)

2. Group of lymphoblasts surrounding a plasma cell (× 1000)

3 Intense mitotic activity in acute lymphoblastic leukaemia (× 1000)

4 Variations in cell size and shape, Rieder cell formation, and a mitotic figure, from a marrow smear of acute lymphoblastic leukaemia (× 1000)

5. Conspicuous cytoplasmic fragmentation of leukaemic lymphoblasts (× 1000)

6 and **7.** Lymphoblasts with characteristic appearance of nucleus filling the cell and early Rieder cell formation (× 1000)

8. Vacuolated cytoplasmic fragment detached from leukaemic lymphoblast (× 1000)

9. Differences in cell size and depth of nuclear staining, with nuclear indentation and cleavage of the Rieder cell type in leukaemic lymphoblasts (× 1000)

10. Leukaemic lymphoblasts with unusually large amount of cytoplasm, but with vacuolation and cytoplasmic fragmentation (× 1000)

CHAPTER 5

THE LEUKAEMIC CELL

I. Cytology and Cytochemistry

In recent years increasing attention has been paid to the detailed structure and function

No attempt will be made to discuss in detail the structure and physiology of normal leucocytes and their precursor cells, nor, indeed, will the vast mass of data concerning the

problems of the nature of leukaemia and the general pathogenesis of the disease. It would be desirable to determine whether the resemblances were superficial, masking an essential disparity, or the differences secondary, imposed upon a basically normal cell, and, since studies in this field can be conducted as easily with human as with rodent material, to decide whether the same answer applies to both man and mouse.

Cytological Studies

The multiplicity of staining procedures and microscopic techniques currently applied to the study of normal and abnormal leucocyte morphology may be conveniently grouped into four classes. the orthodox panoptic staining of dried blood smears with eosin–methylene blue mixtures, more specifically histochemical techniques, the use of supravital stains and phase-contrast microscopy to study living cells, and, finally, electron microscopy.

PLATE V

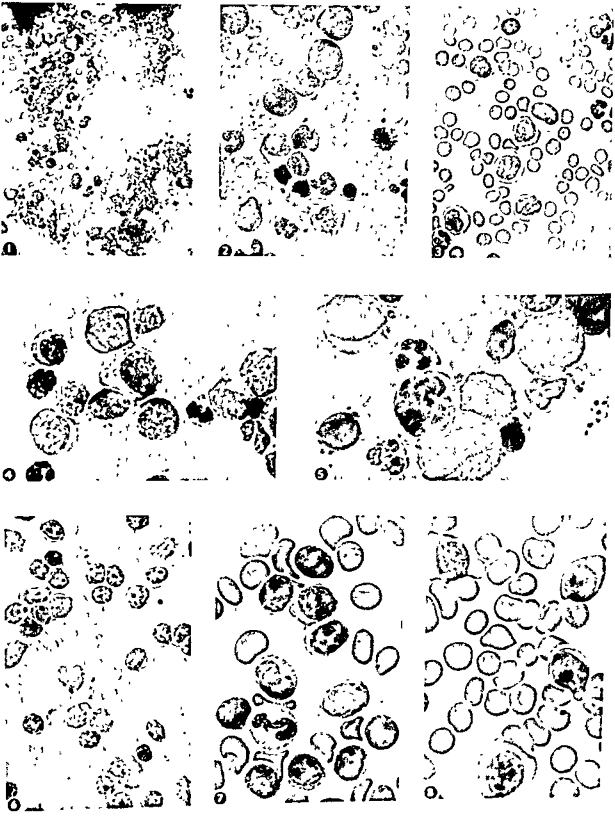
CHRONIC LEUKAEMIA—M.G.G. STAIN

1. Low-power field of peripheral blood in chronic granulocytic leukaemia, showing granulocyte precursors and thrombocytosis. (× 300)
2. [illegible] y granulo- [illegible]
3. [illegible] (× 800 and × 1000)

[illegible] owing smear cells and predominantly [illegible] 600)

[illegible] ukaemia. (× 1000)

PLATE V



To face p. 73

features of individual leukaemic cell cytology are quite commonly seen in leucocyte precursors involved in a leukaemoid proliferation. This parallelism is true of all forms of leukaemia and leukaemoid reactions, including granulocytic, lymphocytic, monocytic and blast cell types. A selection of representative cytological appearances from leukaemic blood and bone-marrow preparations is given in Plates II, III, IV and V, and these illustrations serve also to replace a lengthy textual description of the cytological features revealed in leukaemic cells by panoptic staining.

Auer bodies. In 1906 Auer drew attention to the occurrence of rod-shaped azurophilic bodies, from 1 to 7μ in length, in the cytoplasm of the "large lymphocytes" in acute leukaemia. Subsequent reports on the nature and distribution of these rods have been reviewed by Locquin and Bessis (1949) and Ackerman (1950), who also described the cytochemical reactions given by the bodies. These structures have been most often encountered in leukaemic myeloblasts and promyelocytes, and early reports of their occurrence in lymphoblasts have not been substantiated. They may, however, be found in monoblasts and promonocytes in both acute monocytic and myelo-monocytic leukaemias (Goodwin, 1934, Hawksley, 1935, Kibler, 1947), and have even been reported, rarely, to occur in mature neutrophil polymorphs (Richter, 1923). There is no doubt that Auer bodies are common and conspicuous in many cases of acute leukaemia, and sometimes can be seen in the early myeloid cells of chronic granulocytic leukaemia, but it is not easy to determine from the literature whether non-leukaemic cells may ever contain these rods, and until this point is settled their presence must be regarded as suggestive rather than diagnostic of leukaemia. Bessis (1956) has pointed out that Auer bodies may occasionally be strikingly evident with

Auer bodies occur in about 10 to 20 per cent of acute myeloblastic and monocytic leukaemias, and in cases showing the rods between 2 and 50 per cent of the leukaemic cells may contain them. In an individual cell more than one Auer body may be seen; two, three or four are not unusual, and sometimes feathery clusters of many rods may be found.

Ackerman (1950) and Bessis (1956) have discussed the nature and genesis of Auer bodies, which appear to be an abnormal form of azurophilic granulation, perhaps produced by coacervation of newly formed granules in certain leukaemic cells whose cytoplasmic constitution favours granule fusion. The earliest forms appear as rounded granules which are thought to acrystalline lozenge-shaped

Rieder cells. , and sometimes erroneously regarded as specifically leukaemic, is an extremely coarse lobular division, with deep clefts separating two, three or four masses of nuclear material, so that a tri- or quadri-foliate appearance is given in thin preparations. Cells with such a nuclear pattern are called Rieder cells.

Leukaemic myeloblasts will often develop this nuclear abnormality when kept in suspension between slide and coverslip for a few minutes, but normal cells sometimes do likewise and the change is probably an early degenerative phenomenon. Primitive cells of any cytological variety may manifest this nuclear peculiarity Examples are shown in Plates II and IV.

These will be discussed in turn, with a preliminary account of the technical principles and potentialities of each method and a comparative description of the findings in non-leukaemic and leukaemic leucocytes

General panoptic differential staining

The eosin–methylene blue mixtures of variable composition, usually dissolved in methanol, and often referred to as the "Romanowsky" group of stains, occupy a deservedly unique place among haematological techniques, since they bring into prominence more of the characteristic features of blood cells than does any other staining method. Three principal modifications are in common use today; the most widely popular and successful is that of Pappenheim, in which the May–Grünwald and Giemsa mixtures are used successively, and the descriptive cytological details below are derived from preparations stained in this way. The other two panoptic stains, those of Leishman and Wright, are commonly employed in routine laboratory practice in Great Britain and the U.S.A. respectively. They have the advantage of relative simplicity in general use, but do not provide so brilliant a stain, particularly of the various cytoplasmic granules of leucocytes, as results from the Pappenheim method. The mode of action of these mixtures is extremely complex, and probably depends upon partial dissociation of the components after dilution with water. The resulting intensity of staining will vary according to the extent of dissociation and the nature and quantity of the derived dyes, which may include two methylene azures, in which one or two of the methyl groups of methylene blue are replaced by hydrogen, and a further transformation product of the second azure, Bernthsen's violet.

reproducible and consistent colouring of blood cells, the mechanism is not open to any simple histochemical interpretation.

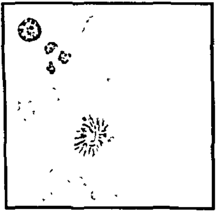
The successive use of May–Grunwald and Giemsa stains gives particularly clear results, since the first emphasizes specific granules, while the second, by virtue of its added content of methylene azures, stains azurophilic granules sharply and is a better nuclear stain. The general effects for all leucocyte components are therefore complementary.

Comparison of leukaemic and non-leukaemic cells. While the blood cells in leukaemia may sometimes be indistinguishable from normal as far as the cytological characters of individual cells stained by the May–Grunwald-Giemsa method are concerned, abnormalities are frequent and striking in most cases, particularly among less-differentiated cells. Early workers emphasized alterations in nuclear-cytoplasmic ratio, variations in general cell size, abnormal size and prominence of nucleoli, discrepant maturation of nucleus and cytoplasm, mitotic abnormalities and changes in chromosome number. A tendency to easy disruption of cells, pycnotic nuclear degeneration, cytoplasmic vacuolation and irregularity of cell outline may also often be observed. That such changes are present in leukaemia is certainly true; that they are specifically leukaemic and represent a recognizable neoplastic change in the leucocyte is more questionable. A sufficiently powerful proliferative stimulus of whatever kind may lead to very comparable abnormalities, and although the overall picture in leukaemoid reactions is often clearly different from that in frank leukaemia, the supposedly specific

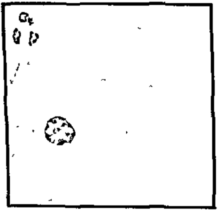
PLATE VI

FEULGEN REACTION. NORMAL BONE MARROW AND LEUKAEMIC BLOOD

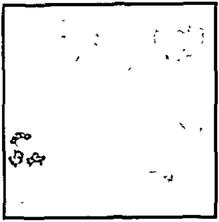
1. Normal bone marrow



2 Acute myeloblastic leukaemia

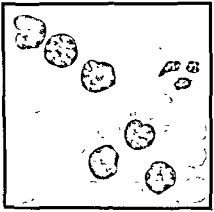


3 Acute lymphoblastic leukaemia



4. Acute monocytic leukaemia

5 Chronic granulocytic leukaemia



6. Chronic lymphocytic leukaemia

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Cytochemical staining

By the use of cytochemical methods an attempt is made to reveal the chemical composition of cells and tissues, usually by the development of a colour reaction, without causing the destruction of the tissues or damaging the cells so much that they become unrecognizable. Perhaps the earliest collected cytochemical observations were made by Raspail in 1830, but for the next hundred years this approach to the study of cells was neglected, save for occasional isolated experiments Most of the sporadic contributions to the subject made during this century of neglect were reviewed by Pearse (1951), but although they included the introduction of the Prussian-blue method for demonstrating iron (Perls, 1867), the use of Sudan III to stain fat (Daddi, 1896), and the detection of calcium in tissues (von Kossa, 1901), it was still possible for Lison in 1936 to regard the subject of his book Histochimie animale as a new science

Since the publication of Lison's excellent monograph the techniques of histochemistry have expanded and multiplied enormously and they have been increasingly applied to problems of pathology and physiology Chemical colour reactions now permit the characterization and differentiation of proteins, lipids, carbohydrates, enzymes, and inorganic substances in their natural situations in the cell cytoplasm or nucleus (Glick, 1949). The publication of the *Journal of Histochemistry and Cytochemistry* since 1953 demonstrates the rapidity with which new information is accumulating on the intimate cellular chemistry of tissues General discussions of the multiplicity of technical methods and the interpretation of results may be found in the books of Lison (1953) and Pearse (1953), but we shall be concerned here only with those cytochemical methods which have been applied to the study of leucocytes, and, in particular, the cells of leukaemia They include the Feulgen reaction, the use of ribonuclease and microspectrophotometry in the study of nucleic acids, the demonstration of lipids by Sudan black B and by Baker's method, the periodic acid-Schiff reaction and other procedures for glycogen, and various peroxidase, phosphatase and other enzyme reactions

The Feulgen reaction. In 1924 Feulgen and Rossenbeck described a specific application of the Schiff test for aldehydes to the histochemical demonstration of desoxyribonucleic acid (DNA). The chemistry of the reaction has been discussed by Stacey and others (1946), and depends upon the liberation of free pentose aldehyde groups from the DNA after hydrolysis with warm hydrochloric acid The free pentose aldehyde groups combine with Schiff's reagent (leucobasic fuchsin, a rosaniline–pararosaniline mixture decolorized by excess of sulphurous acid) to produce a brilliant magenta-coloured substance. To be DNA, the substance responsible for a positive Feulgen reaction must give negative results unless previously hydrolysed with hydrochloric acid

The application of this reaction to haematology has been considerably studied in recent years. Pittaluga and Bessis (1944) used the reaction to investigate the structure and function of the nucleoli in a variety of normal and pathological cells, including primitive haemopoietic cells. They found that the nucleolar substance never gave a positive Feulgen reaction, but stood out as a sharply negative area surrounded by a clearly positive "perinucleolar crown" which in its turn was readil[illegible] m the paler but still positive nuclear chromatin netw[illegible] primary blood cells during the proc[illegible] [illegible]ould be clearly observed. La Cour (19[illegible] [illegible]nd mitotic abnormalities in bone-marrow

cells by the use of the Feulgen stain. He observed a contrast in DNA content between proerythroblast chromosomes and promyelocyte chromosomes at metaphase, the former staining more deeply. In pernicious anaemia the promyelocyte chromosomes were almost negative and much longer and thinner than normal. This appearance was thought to be due to a deficiency of DNA leading to incomplete spiralization. Proerythroblasts and early megaloblasts, on the other hand, showed an excessive charge of DNA with accompanying over-spiralization. Such over-spiralized chromosomes were frequently involved in irregular division, and multipolar spindles and chromatid bridges at anaphase were common. These associations between mitotic abnormalities and nucleic acid content of chromosomes as revealed by the Feulgen reaction were also discussed by Discombe (1948) who compared results obtained with the Feulgen technique and Romanowsky stains, and concluded that chromosomes contained a layer of DNA absorbed on the spiral, with another layer of basophilic material superficial to this. The latter layer was dissolved away in the Feulgen procedure, but coloured by Romanowsky stains.

Rheingold and Wislocki (1948) applied the reaction to normal blood and bone-marrow cells, and Gardikas and Israels (1948) and Hayhoe (1951) discussed the value of the stain in cytological problems in clinical haematology. Nucleated cells of both leucopoietic and erythropoietic tissue take up stain with an intensity proportional to the degree of maturity of their nucleus. Immature cells have a relatively low concentration of nuclear DNA and stain palely, while mature cells have more DNA and give a deep nuclear stain. Nucleoli in primitive cells are unstained and show up more clearly than in Romanowsky preparations. A perinucleolar zone of intensely staining chromatin can often be seen. With increasing maturity of the cells the depth of staining increases, the chromatin pattern becomes coarser, and the nucleoli are lost, so that mature nucleated cells show a very [illegible] cell structure [illegible] istribution, its clear delineation of chromosomes in mitosis, and its sharp demonstration of negatively

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PLATE VII

THE FEULGEN REACTION

[illegible]

one in mitosis, contrasting sharply with the weakly positive leucocyte precursors. (× 1000)

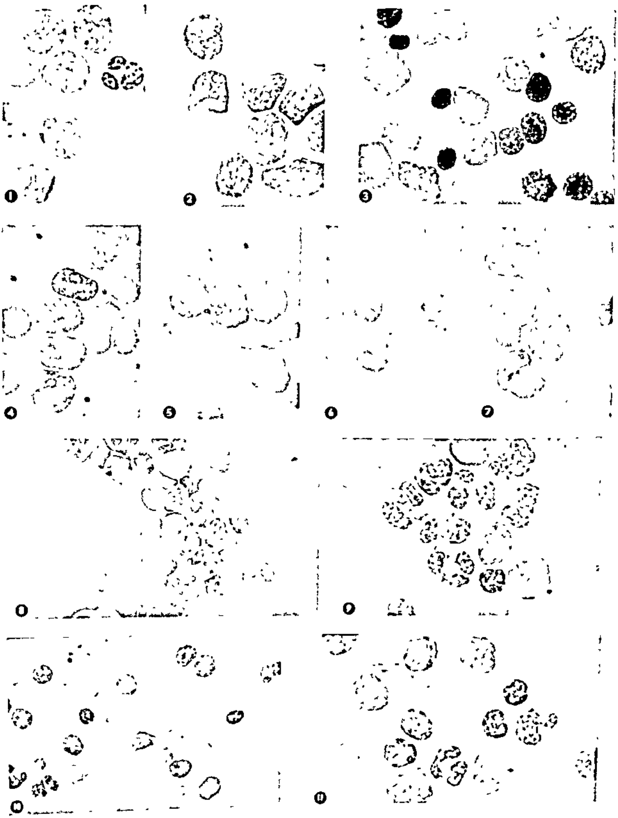
4 and 5. Primitive cells from acute lymphoblastic leukaemia. Nucleoli are few but conspicuous. (× 1000)

6 and 7. Primitive cells from acute monocytic leukaemia, showing variable numbers of nucleoli in [illegible]

8 a [illegible]

10 a [illegible] A single poorly defined nucleolus can be seen in some of less fully differentiated leukaemic lymphocytes

PLATE VII



are not specifically leukaemic, and merely reflect the presence of young, actively proliferating cells.

Ultra-violet spectrophotometry of nucleic acids. An ingenious though elaborate technique to determine the location and concentration of DNA and RNA was introduced by Caspersson and his colleagues (Caspersson, 1936; Caspersson and Santesson, 1942; Caspersson, 1950, 1955). The basis of this method lies in the intense absorption of ultra-violet l

acids.

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marrow cells at different stages of maturity with regard to nucleic acids, proteins and haemoglobin, using their respective maximum absorptions at wavelengths 2,600 Å (for nucleic acids), 2,800 Å (for the tryptophane and tyrosine content of proteins) and 4,050 Å and 4,360 Å (for haemoglobin). Among the red cell precursors Thorell observed an early phase of rapid growth where the concentration of cytoplasmic ribose polynucleotides was

The nucleolus and cytoplasm of primitive cells showed a high RNA content, with no DNA, and the Feulgen positive chromatin and chromosomes contained DNA but not RNA. In all sites within the cell, proteins were found.

Ultra-violet microscopy, like the enzymatic and specific staining techniques, has not revealed any characteristic abnormality of DNA or RNA distribution in leukaemic cells. Accurate quantitative comparisons of the polynucleotide content of different cells cannot yet be achieved, since the optical and physico-chemical properties of cytological material are not yet sufficiently understood (Thorell, 1952; Brachet, 1957), so that the results at present available do not exclude the possible existence of subtle differences in the nature, quantity and distribution of nucleic acids in leukaemic cells; gross differences only are excluded.

Demonstration of glycogen. Neukirch, in 1910, applied Best's carmine method of staining glycogen to human neutrophil polymorphonuclear leucocytes present in blood and inflammatory exudates. He also studied the effect of iodine staining on this material. Both methods disclosed the presence of granules in the cytoplasm of these leucocytes. This granular material was soluble in salivary amylase, and Neukirch concluded that it must be glycogen or a closely related carbohydrate. Stahl, Horstmann and Hilsnitz (1925) *confirmed the existence of iodophilic granules in* granulocytes and found them also in lymphocytes and erythroblasts.

staining nucleoli in contrast to the surrounding intensely positive nucleolus-associated chromatin. The method might therefore be expected to give information on mitotic and nucleolar abnormalities in leukaemia with more precision than the Romanowsky stains. The varieties of mitotic disorder observed by Koller (1947) in various tumours, including changes in chromosome structure arising from defective chromosomal separation at anaphase, alterations in the number of chromosomes, and abnormalities of the mitotic spindle, may all be clearly depicted in Feulgen preparations of leukaemic blood and bone marrow, but, once again, no specifically leukaemic morphological change has been detected. All these abnormalities are met with in non-leukaemic proliferative disorders of haemopiesis, and are, indeed, more conspicuous in the erythroblasts of pernicious anaemia and in the granulocyte precursors in some leukaemoid reactions than in the leucocyte precursors of leukaemia. Examples of Feulgen staining are shown in Plates VI and VII.

The Feulgen reaction is also used in combination with ultraspectrophotometry to assist in the differentiation of desoxy- and pentose nucleotides in the study of nucleoprotein metabolism in haemopoietic cells.

Further methods for cytochemical detection of nucleic acids. Certain basic dyes combine readily with the phosphoric acid groups of nucleic acids and may be used to demonstrate either desoxyribonucleic acid (DNA) or ribonucleic acid (RNA). A valuable combination of dyes is a mixture of methyl green and pyronine, since methyl green stains only DNA while pyronine is specific for RNA The respective specificities of these dyes may be confirmed by the parallel use of purified nucleases; smears exposed to desoxyribonuclease no longer stain with methyl green (or with the Feulgen reagents), while those treated with ribonuclease no longer stain with pyronine (Brachet, 1940, 1942). By the use of methyl green–pyronine mixtures applied to untreated and ribonuclease-digested smears, together with parallel use of the Feulgen reaction, the distribution of DNA and RNA in haemopoietic cells has been determined.

The cytoplasmic basophilia of primitive blood cells, seen in Romanowsky-stained preparations, is due to the presence of RNA, which stains strongly with pyronine and is selectively destroyed by ribonuclease. The nucleoli of young cells also contain RNA and stain with pyronine. White (1947) concluded that the existence of high concentrations of RNA in the cytoplasm and nucleoli of young haemopoietic cells appeared to be closely related to their capacity for proliferation and their ability to synthesize specific cytoplasmic constituents. The presence of RNA has also been demonstrated by these methods in the cytoplasm of lymphocytes and plasma cells (Rheingold and Wislocki, 1948), in neutrophilic granules (Berghe and Hofman, 1945), in Auer bodies of leukaemic myeloblasts (Ackerman, 1950) and in leucocytic toxic granulations in degenerating cells (Jackson, 1954).

Desoxyribonuclease is less used than ribonuclease, since the Feulgen reaction provides a convenient parallel to methyl green in the demonstration of DNA. When methyl green is used the pattern of positivity is confined to the nucleus, and closely resembles that shown in Feulgen preparations, but while the Feulgen reaction is specific to DNA of both high and low polymer type, methyl green stains only highly polymerized DNA (Kurnick, 1952; Vercauteren, 1951).

The immature and hyperplastic marrow state in leukaemia is associated with increased nucleic acid content of the cells, with RNA values particularly raised, but these changes

granulocyte precursors, r
colouring, usually faint, bı
like that seen in myelocyt
granulocytes, but P.A.S.-positive granules can often be demonstrated *in the cytoplasm.* They may be fine or coarse but are usually discrete, varying in number from two or three to about forty, and often arranged in a ring or crown around the periphery of the cell. Monocytes appear normally to contain small amounts of finely scattered glycogen. Platelets contain much P.A.S.-positive material in two forms: the one finely scattered, lightly staining and peripherally distributed, and the other densely clumped, heavily positive and centrally situated. Megakaryocytes show dense staining over and around the nucleus, and fine granules throughout most of the cytoplasm.

Most of the earlier workers did not find any gross differences from normal in the glycogen content of blood and marrow cells in pathological conditions, apart from some increase in granulocyte glycogen in infective states. Gibb and Stowell (1949), for example, reported that leukaemic granulocytes, lymphocytes and monocytes showed a similar positivity to

encountered in bone-marrow and lymph-gland cells from lymphocytic leukaemia. The authors claimed that this phenomenon was not paralleled in the normal lymphocyte series, where increasing glycogen content occurred with increasing maturity, and must therefore be linked with the leukaemic transformation. A high glycogen level in lymphocytes is not, however, peculiar to leukaemia and the application of semiquantitative scoring methods in the assessment of P.A.S. positivity in the lymphocytes in a variety of lymphoproliferative diseases has demonstrated high levels in lymphosarcoma, Hodgkin's disease and other non-malignant lymphocytic proliferations. As a result of such studies, Mitus and his associates (1958*a*) suggested that the glycogen increase was not specifically related to leukaemia but was a non-specific consequence of increased metabolic and proliferative activity in the lymphocytic system. Quaglino and Hayhoe (1959*a*) further demonstrated that a close relationship existed between the level of P.A.S. scores and the effect of treatment in chronic lymphocytic leukaemia. Patients, previously untreated and displaying

richer in glycogen than more mature lymphocytes, although nucleolated lymphosarcoma cells and the lymphoblasts of acute lymphoblastic leukaemia often showed very strong positivity, either in the form of several concentric rings of coarse granules or as heavy blocks of glycogen. Since myeloblasts and promyelocytes from acute leukaemia were substantially negative, showing at most a diffuse cytoplasmic tinge, and leukaemic monocyte precursors, though variable in reaction, failed to show heavy blocks of positive material, these authors suggest that the P.A.S. reaction may be valuable in the differentiation of the cytological varieties of acute leukaemia.

The iodine stains used by these early workers demonstrate glycogen or allied substances by a rather imperfectly understood chemical reaction, and cannot be strictly regarded as histochemical stains. The Bauer–Feulgen chromic acid-Schiff cytochemical technique for glycogen was applied to dried blood films by Mancini and Celani Barry in 1941 and 1942 and the results compared with those obtained with iodine and Best's carmine. These authors found the iodine method to be the most simple and sensitive, but they did not demonstrate the presence of glycogen in lymphocytes, monocytes or early granulocytes. Subsequent workers have used the Bauer–Feulgen method, the periodic acid-Schiff (P.A.S.) technique (McManus, 1946; Hotchkiss, 1948), and a chromic acid–silver–methenamine method developed by Gomori (1946), and have obtained fairly consistent results with all methods, although the Bauer–Feulgen technique appears to be rather less sensitive and produces more diffuse non-specific tinting of the background than do the other methods. The P.A.S. stain is probably the most sensitive, but the silver method gives a black precipitate at glycogen sites and provides especially sharp contrast for photography.

The observations recorded here on glycogen distribution in normal and leukaemic cells have been obtained chiefly from P.A.S. preparations (Wislocki, Rheingold and Dempsey, 1949; Gibb and Stowell, 1949; Hayhoe, 1951; Storti, 1952, Astaldi and Verga, 1957; Quaglino and Hayhoe, 1959), and it is appropriate to discuss briefly the chemical basis of the reaction and its specificity.

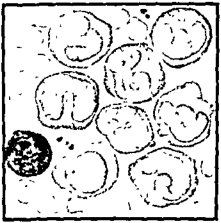
Periodic acid (HIO_4) acts specifically to oxidize the 1–2 glycol grouping (CHOH-CHOH) or its amino- or alkylamino-derivatives to produce a di-aldehyde. The oxidation does not proceed further, and the resulting aldehydes give with Schiff's reagent a substituted dye of magenta colour. According to Hotchkiss (1948), a positive reaction may be given by several classes of naturally occurring carbohydrates, including monosaccharides, polysaccharides, glycoprotein and mucoprotein conjugates, phosphorylated sugars, inositol derivatives and cerebrosides. Substances of low molecular weight are likely to diffuse away during the course of fixation or after oxidation, and glycogen can be distinguished from other positively reacting compounds of high molecular weight by the use of control material subjected to salivary amylase digestion. Compounds such as ribose or desoxyribose nucleic acids and the hydroxyamino acid residues of proteins are chemically substituted so that the free glycol group is not present, and they do not give the reaction.

Positive reactions are localized to the cell cytoplasm. The normal erythropoietic series does not show detectable amounts of glycogen at any stage of cellular development. The myeloid series gives positive results, to a greater or less extent, in all identifiable cells. Neutrophil polymorphonuclear leucocytes contain very large amounts of P.A.S.-positive material, usually in the form of moderately fine granules packed so closely together that no cytoplasmic background can be distinguished. Granules may be seen over the nuclear surface, but they rarely obscure its outline and detail. Neutrophil metamyelocytes and myelocytes contain many glycogen droplets, though not so many as in mature polymorphonuclears. The granules show variations in size and arrangement, being sometimes relatively few and very coarse, but more often numerous and fine. Eosinophil granulocytes have a very distinctive appearance in P.A.S. preparations; the large specific eosinophil granules remain unstained and sharply outlined against a background of diffusely positive intergranular cytoplasm. Basophil cells show a coarse cytoplasmic granularity. Early

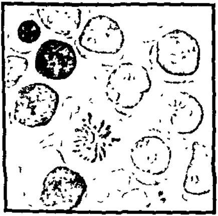
PLATE VIII

PERIODIC ACID-SCHIFF REACTION. NORMAL BONE MARROW AND LEUKAEMIC BLOOD

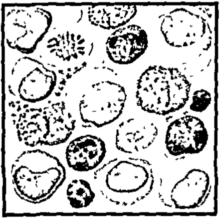
1. Normal bone marrow



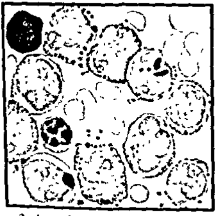
4. Acute monocytic leukaemia



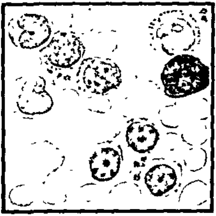
2. Acute myeloblastic leukaemia



5. Chronic granulocytic leukaemia



3. Acute lymphoblastic leukaemia



6. Chronic lymphocytic leukaemia

The erythroblasts are P.A.S. negative in most forms of leukaemia, but in erythroleukaemia and erythraemic myelosis they commonly show strong diffuse and granular positivity (Quaglino and Hayhoe, 1959*b*).

Ackerman (1950) found that Auer bodies gave a positive reaction with the P.A.S. technique, but salivary digestion greatly reduced the positivity. This observation is unconfirmed.

Representative illustrations of P.A.S. preparations of normal and leukaemic cells are shown in Plates VIII–XI.

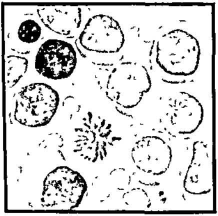
Demonstration of lipids. Early observations on the lipid content of leucocytes were made by Petry (1908), who isolated eosinophil granules and stated that they showed no fat reaction, Sehrt (1927), who described lipid granules in neutrophil polymorphs stained with Sudan III and Nile-blue sulphate, and Bacsich (1935–6), who stained lipid granules in leucocytes with Sudan III but found many samples of this dye unsatisfactory. In 1934 Lison introduced a new inert dis-azo dye, Sudan black B, *which stains neutral fat droplets* very well and has the additional advantage of staining phospholipids and sterols. The first report on the *staining of leucocyte granules with Sudan black B* was by Sheehan (1939), who found that polymorphonuclear leucocytes were filled with deeply stained granules, eosinophil granules appeared to have only a surface layer of lipid, monocytes showed a variable number of granules, while large and small lymphocytes were always quite free from lipid. Myelocytes showed many granules; myeloblasts had usually a small number of obvious granules but were sometimes clear; lymphoblasts contained no granules at all. Subsequent studies by McManus (1945), Wislocki and Dempsey (1946), Wislocki, Bunting and Dempsey (1947) and Bailiff and Kimbrough (1947) provided general confirmation for these findings, although there were some discrepancies over the staining of monocytes, basophils and primitive cells. Most of these workers used methyl alcohol fixation, but Sheehan and Storey (1947) showed that more even and consistent results could be obtained after fixation in formaldehyde vapour and with the use of a Sudan-black solution maintained at a neutral or slightly alkaline reaction, and having a small proportion of phenol added to act as a mordant.

Rheingold and Wislocki (1948) made detailed observations on the sudanophilia of cells of human peripheral blood and bone marrow, and Bloom and Wislocki (1950) compared their results with Sudan black B with those obtained by the use of the acid haematin stain for phospholipids described by Baker (1946). These investigations showed that clearly detectable sudanophilic granules were present in neutrophil leucocyte precursors from the time when specific granules first appeared. These lipid granules stained greyish brown to black and appeared to be identical in number and distribution with the specific neutrophil granules. The granules of eosinophil polymorphs and myelocytes were also sudanophil and showed the darker periphery and clear interior described by Sheehan and other workers. Basophil granules were studied in the blood of patients with chronic myeloid leukaemia and were found to be positive, though ranging in intensity of staining from pale grey to deep black. The larger basophil granules were pale, with a clear centre, while smaller ones stained deeply and appeared solidly black. Since previous workers had differed over the sudanophilia of monocytes, Rheingold and Wislocki studied the blood of patients with a high monocytic count in which these cells were readily identifiable and found the cytoplasmic sudanophilia to be variable, ranging from a few faint dots up to an

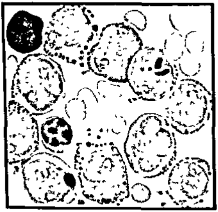
PLATE VIII

PERIODIC ACID-SCHIFF REACTION. NORMAL BONE MARROW AND *LEUKAEMIC BLOOD*

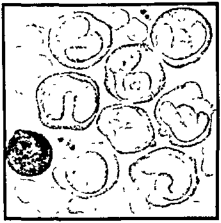
1. Normal bone marrow



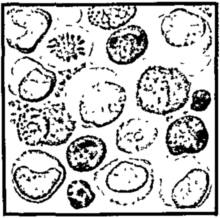
2. Acute myeloblastic leukaemia



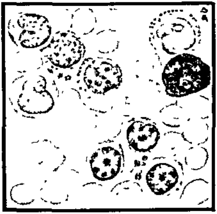
3. Acute lymphoblastic leukaemia



4. Acute monocytic leukaemia



5 Chronic granulocytic leukaemia



6. Chronic lymphocytic leukaemia

To face p.

PLATE IX

PERIODIC ACID-SCHIFF (P.A.S.) REACTION (A)

In this plate and in plates X and XI a number of duplicated identical fields, the first stained by the May–Grunwald–Giemsa (M G G) technique, and the second by the P A S method, are shown Smears were first stained with the M G G method and suitable fields chosen and photographed The specimens were then submitted to the P A S staining procedure, during which the Romanowsky dyes were removed by the periodic acid P A S positivity after this procedure was found to be entirely comparable with that of previously unstained smears After the smears had been lightly counterstained with haematoxylin the fields previously photographed were again found and rephotographed, thus allowing a direct comparison between cytological appearances after M G G and P A S staining to be achieved

1 and **2.** Duplicate M G G and P A S -stained fields from acute myeloblastic leukaemia The primitive cells are uniformly negative (× 1000)

3, 4 and **5.** Cells from P A S -stained blood smears of three further patients with acute myeloblastic leukaemia In each case the myeloblasts show a negative reaction, positive granules being present in a lymphocyte (in **3**), a myelocyte and a metamyelocyte (in **4**) and a mature polymorph (in **5**) (× 1000)

6 and **7.** Duplicate M G G - and P A S stained fields from acute lymphoblastic leukaemia Conspicuous rings of positive granules are present in many of the lymphoblasts, and positive reactions are seen in disrupted cytoplasmic fragments (not to be confused with platelets, which are also positive, but not present in these fields) (× 1000)

8, 9 and **10.** Further P A S stained preparations from different patients with acute lymphoblastic leukaemia, illustrating the variable, but often coarse, annular positivity, and the occasional presence of dense positive blocks, as in **10** This last feature has been encountered most often in childhood leukaemia (× 1000)

PLATE IX

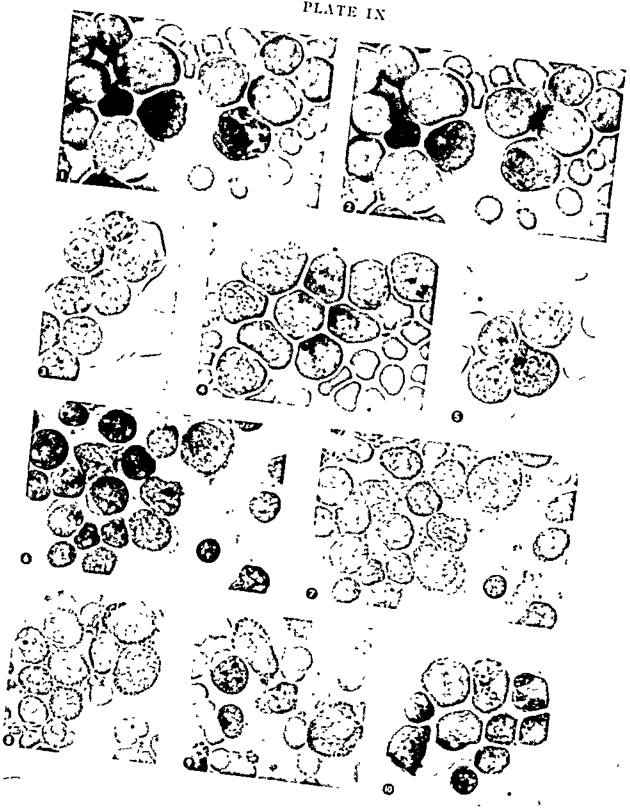


PLATE IX

PERIODIC ACID-SCHIFF (P.A.S.) REACTION (A)

In this plate and in plates X and XI a number of duplicated identical fields, the first stained by the May–Grunwald–Giemsa (M G G) technique, and the second by the P A S method, are shown Smears were first stained with the M G G method and suitable fields chosen and photographed The specimens were then submitted to the P A S staining procedure, during which the Romanowsky dyes were removed by the periodic acid P A S positivity after this procedure was found to be entirely comparable with that of previously unstained smears After the smears had been lightly counterstained with haematoxylin the fields previously photographed were again found and rephotographed, thus allowing a direct comparison between cytological appearances after M G G and P A S staining to be achieved

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PLATE X

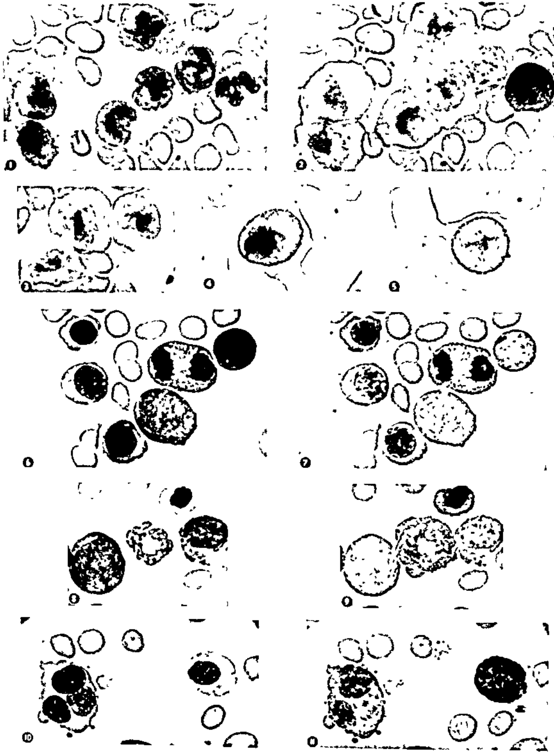

PLATE X

P.A.S. REACTION (B)

1 and **2.** Duplicate M G G - and P A S -stained fields from acute monocytic leukaemia The monocytes in this case were negative, although the single polymorph in the field shows strong cytoplasmic positivity (× 1000)

3, 4 and **5.** Leukaemic cells from three further cases of acute monocytic leukaemia, showing variability in the P A S reaction of monocyte precursors, *from faint granular positivity in* **3** *to moderately strong reactivity* in **4** and coarse peripheral positivity in **5.** (× 1000)

6 an [illegible]

[illegible] *clearly shown* (× 1000)

obvious particulate dusting with coarser lipid granules. These fine dots, and similar ones seen in myeloblasts, lymphoblasts and megakaryocytes stained with Sudan black B, were more distinctly stained by Baker's method and were presumed to be mitochondrial phospholipid. Lymphocytes were uniformly negative.

was regarded as of value in differentiating the forms of acute leukaemia. In a similar study, Hayhoe (1953) found the myeloblasts of acute myeloid leukaemia to possess many very fine and lightly stained greyish-brown dots and rods in the cytoplasm, particularly conspicuous in the juxta-nuclear areas and usually concentrated centrally between the lobes of the nucleus in paramyeloblasts. These, and comparable granules in the primitive cells of other cytological varieties of acute leukaemia, were presumably mitochondria and did not differ significantly from those to be seen in normal precursor cells. In chronic myeloid leukaemia most granulocytes were indistinguishable in Sudan-black reaction from normal cells of similar maturity, but in several peripheral-blood smears a small proportion—some two or three per cent—of mature polymorphs showed no stained granules; no comparable observation of granule-free polymorphs was made in normal blood or marrow. A more striking deviation from the normal pattern was seen in chronic lymphatic leukaemia. Many mature lymphocytes were negative and almost invisible in uncounterstained Sudan black B preparations, but a variable proportion, about 10 per cent in one case, 50 per cent in another, and none at all in a third, showed a dark, finely granular, positive reaction in the narrow cytoplasmic rim of the cell. Lymphocytes in normal blood and in a variety of non-leukaemic pathological conditions examined were always negative, and this phenomenon of lymphocytic sudanophilia in chronic lymphatic leukaemia may perhaps be peculiar to the disease, although the cells of lymphocytic leukaemoid reactions have not been studied in this regard.

Locquin and Bessis (1949) and Ackerman (1950) found Auer rods to be strongly sudanophilic.

Results of Sudan black B staining of normal and leukaemic cells are illustrated in Plates XII and XIII.

Peroxidase reactions. The presence of a peroxidase enzyme system in leucocytes can be demonstrated by the use of an oxidizable substrate having brightly coloured oxidation

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PLATE XI

P.A.S. REACTION (C)

... leukaemia, showing slight or moderate granular positivity in the cytoplasm of many lymphocytes. The strongly positive reaction in a polymorph (in 8) contrasts with the weaker reaction of polymorphs in chronic granulocytic leukaemia (see 2). (× 1000)

PLATE XI

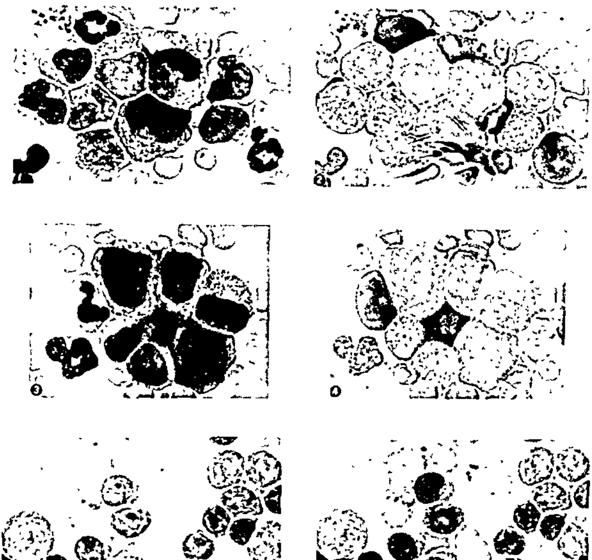

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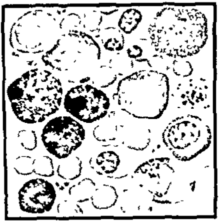
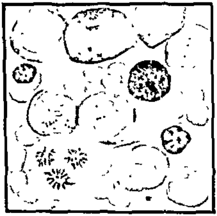
PLATE XII

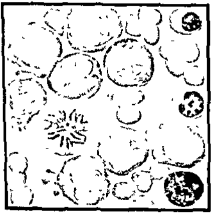
SUDAN BLACK B STAIN. NORMAL BONE MARROW AND LEUKAEMIC BLOOD



1. Normal bone marrow

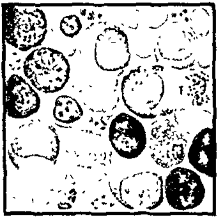


2. Acute myeloblastic leukaemia

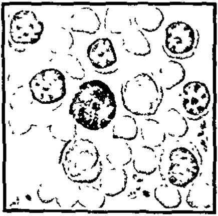


3. Acute lymphoblastic leukaemia

4. Acute monocytic leukaemia



5. Chronic granulocytic leukaemia



6. Chronic lymphocytic leukaemia

products. The most commonly used and most satisfactory of the available substrates until recent years has been benzidine, which yields blue or brown compounds on oxidation. It was first introduced by Graham (1918) for the study of blood films, and his method forms the basis of most peroxidase reactions used in haematology today, although many modifications with respect to fixation and mordanting have been employed by different investigators, and since benzidine itself is a carcinogenic substance it is now being replaced by *o*-tolidine. Methods which allow the use of a Romanowsky counterstain are especially valuable, since the cells can be more easily identified, and the illustrations in Plate XIV are from preparations counterstained in this way after peroxidase staining by a modification of the Graham–Knoll technique, in which *o*-tolidine replaces benzidine (Quaglino and Flemans, 1958).

All granulocytes give a positive cytoplasmic reaction and appear crowded with yellowish-brown granules. Lymphocytes are negative, and monocytes contain a variable number of granules, usually finer and more scanty than those in granulocytes. The method has been regarded as of value in differentiating myeloblasts from lymphoblasts, since myeloblasts often show some few peroxidase granules whereas lymphoblasts are uniformly negative, but the distinction cannot always be made by this means because the youngest myeloblasts are also peroxidase-negative. Auer bodies are peroxidase positive.

Study of the blood and marrow cells in leukaemia has not hitherto revealed any specific abnormality of peroxidase distribution.

The relation between specific, P.A.S.-positive, sudanophilic and peroxidase granules. The general distribution of all these granules is roughly comparable. Sudanophilic granules are almost certainly identical with the specific granules of Romanowsky-stained smears since their occurrence, number and distribution in all cells of the granulocytic series run closely parallel A further corroborative observation is that the specific granules of a Leishman-stained preparation become obscured if, after short exposure to Leishman's stain, the slide is stained with Sudan black B. Again, counterstaining Sudan-black preparations with Leishman's stain reveals no specific granules distinct from the sudanophilic granules. Sudanophilic granules are, however, larger than specific granules, and this difference was discussed by Discombe (1946), who concluded that the discrepancy was optifactual, resulting from the absorption of one of the Leishman azures, probably Bernthsen's violet, on the surface of the lipid granule, with the production of a discoid image of smaller diameter than the granule itself when light from the condenser was focused by the granule.

noted by Sehrt (1927), who concluded that the oxidase reaction of blood cells was proportional to their lipid content, and by Lison (1936*b*), who thought it possible that the oxidase positivity depended less on the presence or absence of oxidases, than on the existence in the cell of lipids capable of dissolving and retaining oxidation products.

A careful study of the relationship of leucocyte sudanophilia to leucocytic oxidase and peroxidase reactivity was reported by Lillie and Burtner (1953). Exposure of blood smears to a wide variety of physical and chemical procedures potentially active in extracting

PLATE XIII

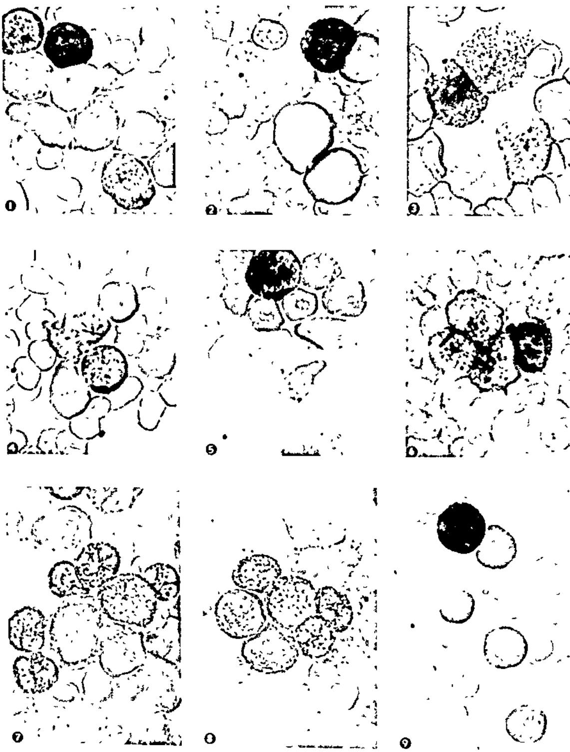


PLATE XIII

SUDAN BLACK B

(Preparations lightly counterstained with haematoxylin with the exception of those shown in 7 and 8)

1. Cells from acute myeloblastic leukaemia, showing negative or faintly positive reaction in myeloblasts, clear positivity in promyelocytes and a single strongly sudanophilic polymorph (× 1000)
2. Completely negative primitive cells from acute lymphoblastic leukaemia (× 1000)
3. Cells from acute monocytic leukaemia, showing typical finely scattered positive granules in three primitive cells, a fourth cell being negative (× 1000)
4. Conspicuous positive Auer rods in acute myeloblastic leukaemia (× 1000)
5. Negative lymphoblasts with marked vacuole formation, showing that the vacuoles are Sudan Black negative (× 1000)
6. Group of three monocyte precursors and a polymorph from acute monocytic leukaemia, illustrating numerous coarsely positive Auer rods of various shapes and sizes (× 1000)

7 and 8 Granulocytes from chronic granulocytic leukaemia, uncounterstained, showing increasing sudanophilia with increasing cell maturity (× 1000)

9. Negative lymphocytes and a very strongly positive polymorph from chronic lymphocytic leukaemia (× 1000)

lipids or destroying enzymes led these workers to the conclusion that sudanophilia of leucocytes was not dependent on the presence of lipid, but resulted from an unexplained chemical combination of the dye with non-lipid cytoplasmic constituents. The earlier conclusion of Agner (1941) that the benzidine peroxidase and the stable M-Nadi oxidase reactions were essentially similar and due to the presence of a leucocytic peroxidase enzyme system was supported, and the suggestion of Sehrt (1927), Lison (1936*b*) and Gomori (1951) that the "oxidase" reaction was attributable to fatty acid peroxides and not to enzymatic activity was not confirmed.

Despite the discrepant views of different workers as to the precise cellular components responsible for sudanophilia and peroxidase reactivity, these components are probably located in or upon the specific granules This conclusion is supported by the observations of Takikawa and his associates (1957), who isolated leucocyte specific granules, mitochondria and cell nuclei by homogenization and differential centrifugation, and found the peroxidase activity chiefly confined to the granular fraction.

The intracellular location of P.A.S.-positive cytoplasmic material appears to differ from that of specific, sudanophilic and peroxidase granules. In cells of the eosinophil and

and that these could be stained in the same preparation by a modified combination of Sudan black B and P A S. techniques applied successively to the smear. Glycogen granules therefore form a component of the cell cytoplasm separate from the other stainable granules.

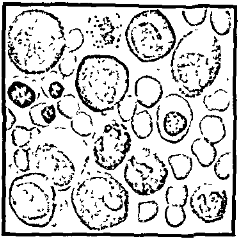
The demonstration of cytochrome oxidase in leucocytes. The labile or G-Nadi oxidase reaction, described by Graff (1916), indicates the presence of the cytochrome-*c*-cytochrome oxidase system. The staining procedure involves the oxidation of a mixture of alpha-naphthol and dimethyl-*p*-phenylenediamine in the presence of this enzyme system with the production of insoluble indophenol blue. The specificity of the reaction is discussed by Pearse (1953).

Hoffmann, Rottino and Stern (1951) applied this method to cells of lymphoid and myeloid tissue from healthy individuals and from patients with leukaemia and Hodgkin's disease. Positive granules were detected in the cytoplasm of all cell types examined; nuclei were invariably quite negative. Lymphocytes contained some 5 to 20 round, deep blue, cytoplasmic granules; polymorphonuclear leucocytes usually showed rather fewer granules; myelocytes were more active than mature granulocytes; monocytes showed the greatest intensity of reaction and showed numerous positive granules. Megakaryocytes and platelets were inactive. Cells from both myeloid and lymphatic leukaemia showed about the same amount of activity as their normal counterparts and no distinctively leukaemic features were identified.

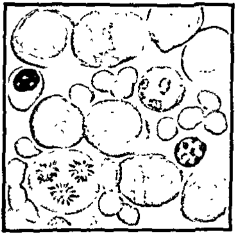
The cytoplasmic particles stained by the G-Nadi reaction correspond in size, distribution and number to particles stained supravitally by Janus green, and presumably are closely related to mitochondria In the experiments of Takikawa *et al.* (1957) with isolated cell components, cytochrome oxidase activity and the closely associated succinic dehydrogenase activity were chiefly concentrated in the mitochondrial fraction of leucocytes.

PLATE XIV

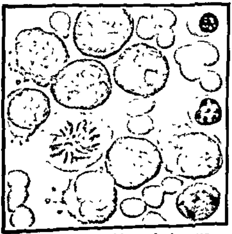
PEROXIDASE REACTION. NORMAL BONE MARROW AND LEUKAEMIC BLOOD



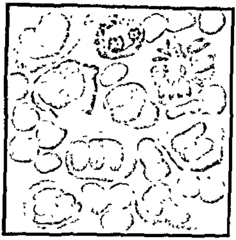
1. Normal bone marrow



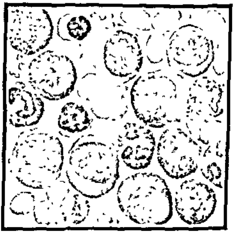
2. Acute myeloblastic leukaemia



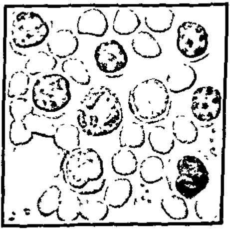
3. Acute lymphoblastic leukaemia



4. Acute monocytic leukaemia



5. Chronic granulocytic leukaemia



6. Chronic lymphocytic leukaemia

ization of alkaline phosphatase by Menten, Junge and Green (1944) and numerous modifications of the original technique have subsequently been proposed (Manheimer and Seligman, 1948; Gomori, 1952; Grogg and Pearse, 1952*a*; Kaplow, 1955). Comparable techniques for the demonstration of acid phosphatase by immediate azo-coupling or post-incubation coupling were described by Seligman and Manheimer (1949), Grogg and Pearse (1952*b*), Burton (1954), Rutenburg and Seligman (1955) and others.

Of all the histochemical staining methods, those for phosphatases, and particularly alkaline phosphatase, have been claimed to reveal the most significant differences between leukaemic and non-leukaemic leucocytes. Most of the initial studies were carried out with the calcium or lead phosphate methods. Wachstein (1946) and Plum (1950) found the alkaline phosphatase content of the cells in chronic myeloid leukaemia to be greatly diminished, in sharp contrast to myelofibrosis, leukaemoid reactions and inflammatory leucocytoses, where the enzyme was unusually abundant in the leucocytes; normal neutrophils were intermediate in activity. These results are in general conformity with those obtained by biochemical analyses (Valentine and Beck, 1951; Beck and Valentine, 1951; Valentine *et al.*, 1952; Wiltshaw and Moloney, 1955), but unfortunately the Gomori–Takamatsu procedure suffers from many drawbacks as a definitive cytochemical method and gives little information on precise intracellular localization of enzyme activity. Indeed, the most striking feature of the reports on both acid and alkaline phosphatase activity in blood and bone-marrow cells studied by the phosphate method has been the variable and contradictory nature of the results. The findings of Wachstein (1946), Rheingold and Wislocki (1948), Rabinovitch and Andreucci (1949), Kerppola (1951), Brodell and Swisher (1954) and Wiltshaw and Moloney (1955) amply illustrate the lack of uniformity as to the cell types reported to show positive staining and the location of reaction sites within individual cells. Many workers have shown that the nuclear staining which often predominates when these techniques are used does not represent phosphatase activity but is an artifact due to phosphate diffusion from some neighbouring site of enzyme activity, and this problem of false positivity applies not only to the nucleus but probably also to other structures within the cell (Martin and Jacoby, 1949; Gomori, 1950*b*; Novikoff, 1951; Johansen and Linderstrøm-Lang, 1953; Gomori and Benditt, 1953; Pearce, 1953).

In view of the difficulties in interpreting results it is scarcely worth while to describe in detail the findings of different investigators who have used these unsatisfactory techniques.

Relatively few reports have appeared on the application of azo-dye methods to the demonstration of acid and alkaline phosphatases in blood and marrow cells. Kaplow (1955) used this technique and found alkaline enzymatic activity confined to neutrophilic granulocytes and localized exclusively in the cytoplasm, but leukaemic cells were not apparently studied.

Hayhoe and Quaglino (1958) studied the leucocyte alkaline phosphatase activity in normal subjects and in a variety of diseases, including all forms of leukaemia, by an azo-dye cytochemical technique, modified from that described by Kaplow, involving fixation in ice-cold formalin–methanol and incubation in a substrate containing alpha naphthyl phosphate, Brentamine Fast Garnet (the diazonium salt of *o*-amino-azotoluene) and a propanediol buffer. The method proved very reliable and satisfactory in regular use and enabled an accurate scoring of cell positivity to be carried out. Positive reactions were substantially confined to the cytoplasm of mature neutrophils, all other cells of the

Leucocytic dehydrogenases. Wachstein (1950) used tetrazolium salts to provide a histochemical demonstration of dehydrogenating enzymes in blood and bone-marrow cells. Hydrogen transfer to these salts converts them from colourless water-soluble substances to highly coloured insoluble precipitates. Better results were obtained by the addition of sodium succinate and other activators to the incubation mixture (Wachstein and Meisel, 1954). Polymorphonuclear leucocytes, lymphocytes, and monocytes contain varying numbers of purple granules indicating sites of dehydrogenase activity. The granules are independent from specific granulation, but probably related to cytochrome oxidase granules, being similarly concentrated in the mitochondrial fraction of isolated cell components.

In infectious diseases and inflammatory states the leucocytes are reported to show increased dehydrogenase activity, but the leucocytes of myeloid leukaemia do not apparently differ in activity from their normal counterparts.

Histochemical demonstration of phosphatases in normal and leukaemic cells. *Acid and alkaline phosphatases, or phosphomonoesterases,* liberate orthophosphoric acid from many alcoholic or phenolic monoesters. They differ chiefly in their widely separated pH optima and in their reactions to certain modifying agents. Magnesium ions, for example, are strong activators for the alkaline enzyme, while almost totally inactive with the acid enzyme. The same is true to a lesser extent of other divalent cations such as Mn^{++}, Zn^{++}, and Co^{++}. Fluorides powerfully inhibit the acid enzyme, while relatively ineffective against the alkaline enzyme. A substantial literature has now accumulated on the histochemistry of phosphatases, although the techniques available until recently were not entirely satisfactory when applied to blood and marrow smears and the results tended to vary and were not easy to interpret.

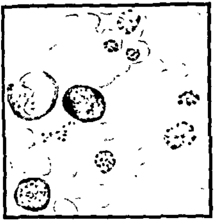
The cytochemical characterization of the phosphatases depends on the formation of an insoluble coloured precipitate at the site where substrate hydrolysis has occurred. Two general techniques have been devised for the demonstration of non-specific phosphatases.

In the first, the enzyme-bearing material is exposed to a suitable phosphomonoester in the presence of ions which are capable of forming an insoluble compound with the released phosphate ions in the selected pH conditions. Gomori (1939) and Takamatsu (1939) independently developed a method for accomplishing this reaction in the case of the alkaline enzyme, and their technique, with minor variations (Wachstein, 1946; Danielli, 1946), has been widely applied in haematology. The method depends on the deposition of insoluble calcium phosphate at sites of phosphatase activity when material is incubated with a glycerophosphate at pH 9 in the presence of calcium ions. Magnesium ions are added to activate the hydrolysis. The precipitated phosphate is rendered visible by conversion into cobalt phosphate followed by conversion of the latter into black cobalt sulphide. Gomori (1941, 1950*a*) employed a similar method for the demonstration of acid phosphatase. At pH 4·7–5, calcium phosphate is soluble, and lead nitrate is therefore added instead of calcium nitrate to the incubating medium. The insoluble lead phosphate precipitated is made visible by conversion into black lead sulphide.

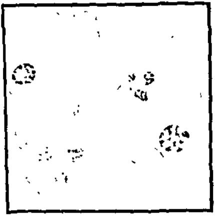
The second technique, applicable with suitable modifications to both acid and alkaline phosphatase demonstration, involves the use of a substrate containing a naphthyl phosphate. As hydrolysis occurs, the liberated naphthol is coupled with a diazotized amine to form an insoluble highly coloured precipitate. This method was introduced for the local-

PLATE XV

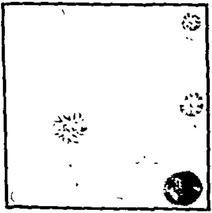
ALKALINE PHOSPHATASE REACTION. NORMAL BONE MARROW AND LEUKAEMIC BLOOD



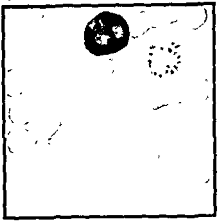
1. Normal bone marrow



2. Acute myeloblastic leukaemia

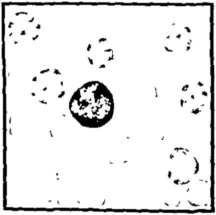


3. Acute lymphoblastic leukaemia



4. Acute monocytic leukaemia

5. Chronic granulocytic leukaemia



6. Chronic lymphocytic leukaemia

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peripheral blood being negative. When individual cell positivity was rated from 0 to 4, and 100 consecutive cell ratings added to give a total score for any blood sample, normal subjects were found to have scores ranging from 14 to 100. Scores much above this range were found in leukaemoid reactions, pyogenic infections and non-leukaemic myeloproliferative states, while in chronic granulocytic leukaemia the scores were almost invariably well below normal.

The superior sensitivity and reliability of the method as compared with the Gomori-Takamatsu technique and the biochemical studies of mixed cell populations revealed further new data.

Repeated scores in chronic granulocytic leukaemia showed a tendency in remitting cases for the leucocyte phosphatase level to return towards the normal range. The change was not invariable, but occurred in most cases. In very full remission an occasional high normal score was found, but such a reversion is rare, the enzyme activity in remission being usually near or within the lower range of normal.

In chronic lymphocytic leukaemia the neutrophil phosphatase scores were always within or above the normal range, while in lymphosarcoma and reticulum cell sarcoma normal or low scores were found. In Hodgkin's disease enzyme activity was always increased during relapse, though returning towards normal in phases of remission.

Studies of acute leukaemia currently in progress show high neutrophil alkaline phosphatase levels in lymphoblastic leukaemia, very low levels in myeloblastic leukaemia, and intermediate scores in monocytic and myelomonocytic leukaemias.

The value of cytochemical phosphatase scoring in differential diagnosis is obvious and its relevance to the concept of myeloproliferative disease (Mitus *et al.*, 1958*b*) will be discussed in Chapter 16, but the fundamental significance of the enzyme variations in leukaemia remains to be determined.

Illustrative fields from alkaline phosphatase preparations in the different varieties of leukaemia are shown in Plates XV and XVI.

Studies of the acid enzyme have not yet given consistent or contributory results.

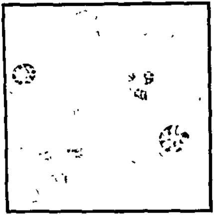
Other enzyme systems demonstrable histochemically. Histochemical methods have been developed for the cellular characterization of several other enzymes. These include 5-nucleotidase, an enzyme capable of hydrolysing nucleotides such as muscle adenylic acid (Wachstein and Meisel, 1952), glucose-6-phosphatase (Chiquoine, 1953), non-specific leucocytic esterases (Gomori, 1953), acetylcholine-esterase (Zajicek, Sylven and Datta, 1954) and beta glucuronidase (Seligman *et al.*, 1954). The capacity of these methods to elucidate patterns of enzyme distribution within leucocytes has not yet been fully explored, and the results so far reported do not reveal any unusual leucocytic activity in leukaemia, but further investigation with these and similar techniques may prove fruitful.

Cytochemistry of inorganic elements. Numerous methods exist for the intracellular demonstration of metals, including iron, calcium, copper, zinc, potassium, lead, bismuth, mercury, and many others, and also for such salts as phosphates, carbonates and chlorides. The available techniques are described and reviewed by Lison (1953), Glick (1949), Pearse (1953) and Gomori (1952) in their texts. Relatively little has been reported on leucocytic metal cytochemistry, either because the methods have proved unsatisfactory for use with blood and bone-marrow smears, or because leucocytes do not contain readily de-

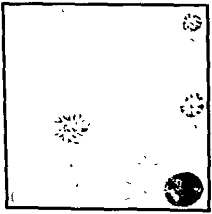
PLATE XV

ALKALINE PHOSPHATASE REACTION. NORMAL BONE MARROW AND LEUKAEMIC BLOOD

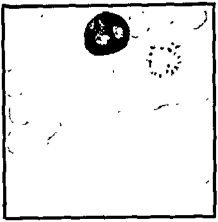
1. Normal bone marrow



2. Acute myeloblastic leukaemia

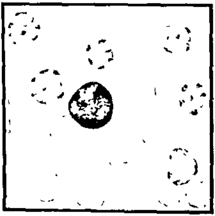


3. Acute lymphoblastic leukaemia



4. Acute monocytic leukaemia

5. Chronic granulocytic leukaemia



6. Chronic lymphocytic leukaemia

peripheral blood being negative. When individual cell positivity was rated from 0 to 4, and 100 consecutive cell ratings added to give a total score for any blood sample, normal subjects were found to have scores ranging from 14 to 100. Scores much above this range were found in leukaemoid reactions, pyogenic infections and non-leukaemic myeloproliferative states, while in chronic granulocytic leukaemia the scores were almost invariably well below normal.

The superior sensitivity and reliability of the method as compared with the Gomori-Takamatsu technique and the biochemical studies of mixed cell populations revealed further new data.

Repeated scores in chronic granulocytic leukaemia showed a tendency in remitting cases for the leucocyte phosphatase level to return towards the normal range. The change was not invariable, but occurred in most cases. In very full remission an occasional high normal score was found, but such a reversion is rare, the enzyme activity in remission being usually near or within the lower range of normal.

In chronic lymphocytic leukaemia the neutrophil phosphatase scores were always within or above the normal range, while in lymphosarcoma and reticulum cell sarcoma normal or low scores were found. In Hodgkin's disease enzyme activity was always increased during relapse, though returning towards normal in phases of remission.

Studies of acute leukaemia currently in progress show high neutrophil alkaline phosphatase levels in lymphoblastic leukaemia, very low levels in myeloblastic leukaemia, and intermediate scores in monocytic and myelomonocytic leukaemias.

The value of cytochemical phosphatase scoring in differential diagnosis is obvious and its relevance to the concept of myeloproliferative disease (Mitus *et al.*, 1958*b*) will be discussed in Chapter 16, but the fundamental significance of the enzyme variations in leukaemia remains to be determined.

Illustrative fields from alkaline phosphatase preparations in the different varieties of leukaemia are shown in Plates XV and XVI.

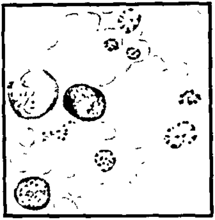
Studies of the acid enzyme have not yet given consistent or contributory results.

Other enzyme systems demonstrable histochemically. Histochemical methods have been developed for the cellular characterization of several other enzymes. These include 5-nucleotidase, an enzyme capable of hydrolysing nucleotides such as muscle adenylic acid (Wachstein and Meisel, 1952), glucose-6-phosphatase (Chiquoine, 1953), non-specific leucocytic esterases (Gomori, 1953), acetylcholine-esterase (Zajicek, Sylven and Datta, 1954) and beta glucuronidase (Seligman *et al.*, 1954). The capacity of these methods to elucidate patterns of enzyme distribution within leucocytes has not yet been fully explored, and the results so far reported do not reveal any unusual leucocytic activity in leukaemia, but further investigation with these and similar techniques may prove fruitful.

Cytochemistry of inorganic elements. Numerous methods exist for the intracellular demonstration of metals, including iron, calcium, copper, zinc, potassium, lead, bismuth, mercury, and many others, and also for such salts as phosphates, carbonates and chlorides. The available techniques are described and reviewed by Lison (1953), Glick (1949), Pearse (1953) and Gomori (1952) in their texts. Relatively little has been reported on leucocytic metal cytochemistry, either because the methods have proved unsatisfactory for use with blood and bone-marrow smears, or because leucocytes do not contain readily de-

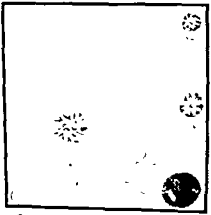
PLATE XV

ALKALINE PHOSPHATASE REACTION. NORMAL BONE MARROW AND LEUKAEMIC BLOOD

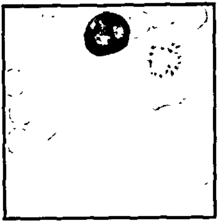


1. Normal bone marrow

2. Acute myeloblastic leukaemia



3. Acute lymphoblastic leukaemia



4. Acute monocytic leukaemia

5. Chronic granulocytic leukaemia

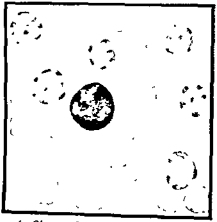


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PLATE XVI

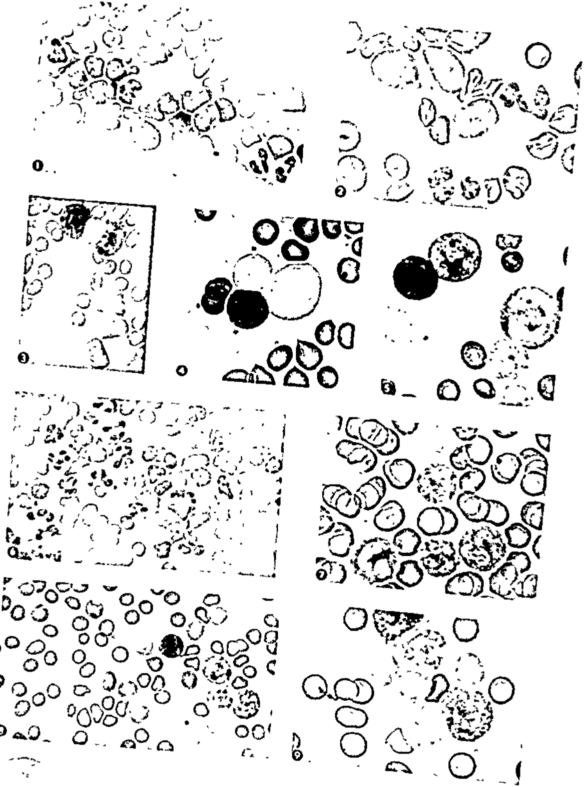


PLATE XVI

ALKALINE PHOSPHATASE REACTION

1 and **2.** Acute myeloblastic leukaemia, showing negative polymorphs. (× 600, × 1000)

3 and **4.** Acute lymphoblastic leukaemia, showing strongly positive polymorphs (× 600, × 1000)

5. Variably positive polymorphs in acute monocytic leukaemia (× 1000)

6. Conspicuously negative polymorphs in chronic granulocytic leukaemia (× 600)

7. Strongly positive polymorphs in leukaemoid reaction (× 1000)

8 and **9.** Variable positivity in polymorphs in chronic lymphocytic leukaemia. (× 600, × 1000)

PLATE XVI

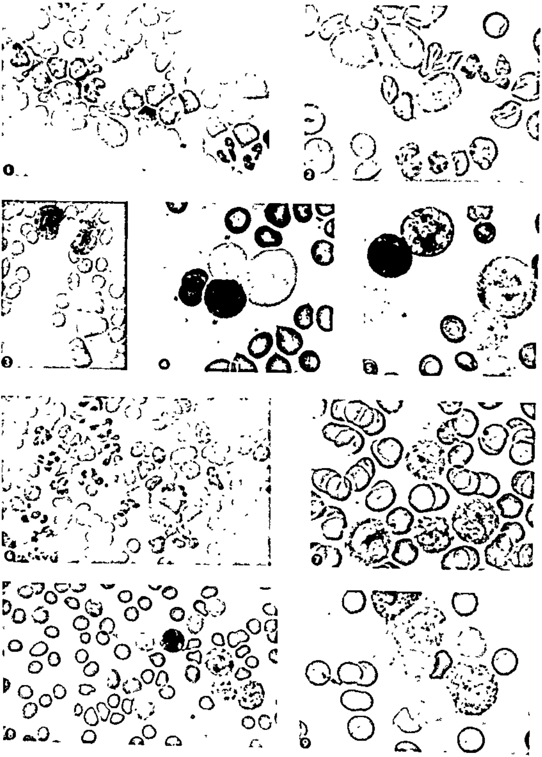


PLATE XVII

PHASE-CONTRAST MICROSCOPY (A)

1. Group of myeloblasts, with variable numbers of nucleoli and scattered mitochondria from acute myeloblastic leukaemia. At the lower left corner are two plasma cells, showing darker, eccentric, nuclei. (× 1800)
2. A typical group of leukaemic myeloblasts. (× 1800)
3. Lymphoblasts, one showing concentration of mitochondria in the juxta-nuclear zone (× 1800)
4. A leukaemic monocyte extending a process round an erythrocyte. (× 1800)
5. Juxta-nuclear mitochondria in a lymphoblast (× 1800)
6. An Auer rod in a leukaemic myeloblast (× 1800)
7. Two monocytes, with much pseudopodia formation, from acute monocytic leukaemia (× 1800)

PLATE XVII

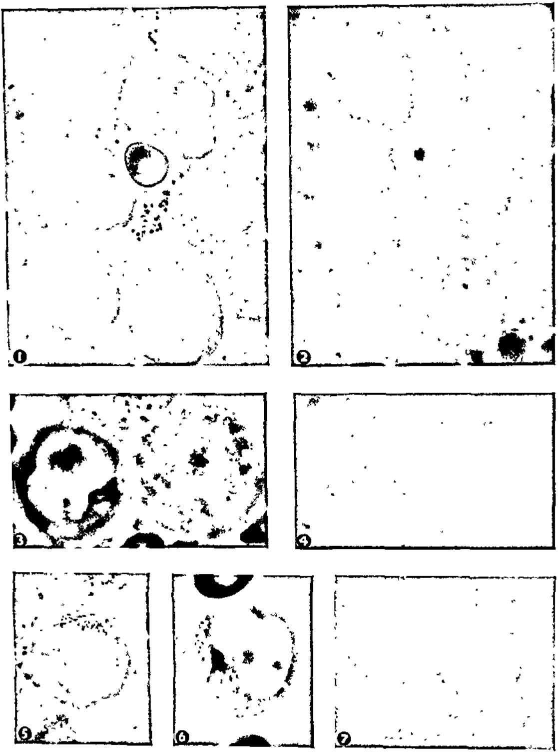
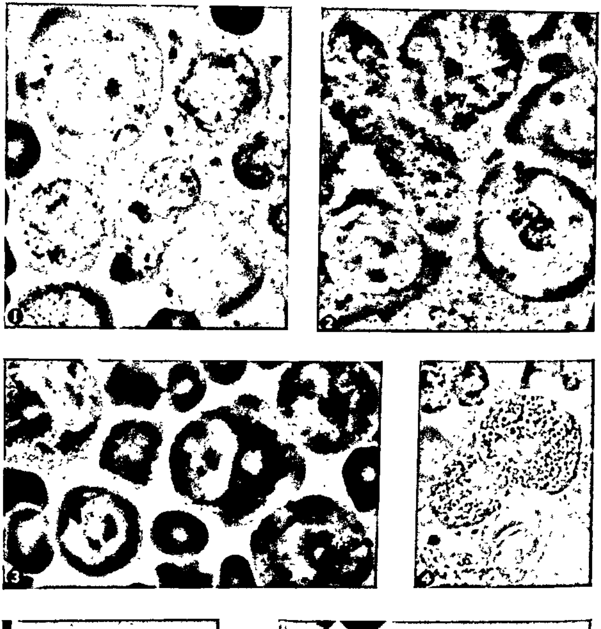


PLATE XVIII



To face p. 89

tectable quantities of stainable inorganic substances. Amann and Wolff (1956), however, reported that satisfactory histochemical staining of zinc and copper in leucocytes could be achieved by several methods and this may be a profitable field of study since quantitative biochemical assays have disclosed a marked reduction in zinc and copper content of leukaemic leucocytes (Vallee and Altschule, 1949; Amann and Wolff, 1956).

Phase-contrast microscopy of living leucocytes

The method of phase-contrast microscopy enables the observer to study living cells under optical conditions good enough to reveal such intracellular components as nuclear chromatin, chromosomes, nucleoli, mitochondria, the centrosome and specific cytoplasmic granules in clear detail, and free from artefacts due to fixation and staining. The principle of the method lies in the conversion of invisible phase changes in light-waves passing through colourless, unstained objects to visible intensity or amplitude changes. The final image of a stained object seen through a microscope results from interference between direct and indirect or diffracted light-rays leaving the object. The direct and diffracted rays from a colourless object can be made to interfere in a similar way, with the production of a visible contrasting image, if a fresh phase difference of a quarter of a wavelength is introduced between them during their passage through the optical system. If a hollow cone of light from an annular diaphragm in the front focal plane of the substage condenser is passed through the object, the direct image will form an annulus in the back focal plane of the objective. At this site is placed a transparent disc or phase plate, having an annular groove arranged to coincide with the direct image and of such a depth that the direct rays emerging from the groove differ from the indirect rays emerging from the remainder of the phase plate by a newly introduced phase change of the required quarter of a wavelength.

The optical theory upon which the method of phase-contrast microscopy is based is complicated and calls for elaborate mathematical treatment (Zernike, 1934, 1942; Françon, 1952; Osterberg, 1955; Barer, 1956). It would be inappropriate to discuss the optics further here, except to point out that phase-contrast microscopy appears to provide a limit of resolution comparable to that of an objective of equivalent numerical aperture used with ordinary illumination, while, with good contrast, individual particles below the theoretical limit of resolution may be clearly visible.

PLATE XVIII

PHASE-CONTRAST MICROSCOPY (B)

1. A clump of red cell precursors, including a proerythroblast, from the marrow of a patient with chronic granulocytic leukaemia in remission under chemotherapy. (× 1800)
2. Various granulocytes from the marrow in chronic granulocytic leukaemia, illustrating formation of pseudopodia and distortions of cell shape during phases of active motility. Nucleoli, cell granules, and vacuoles are well shown. (× 1800)
3. Granulocytes from chronic granulocytic leukaemia, showing the clear juxta-nuclear area of archoplasm among the cytoplasmic granules. (× 1800)
4. An eosinophil polymorph thrusting between neighbouring cells. (× 1800)
5. Large and small lymphocytes from chronic lymphocytic leukaemia. (× 1800)
6. Mature small lymphocytes from chronic lymphocytic leukaemia. (× 1800)

Since the first reports of phase-contrast microscopy of blood and marrow cells (Jones, 1948*a* and *b*), many haematologists have used the method to study the structure and movements of living normal and pathological cells. Bessis, who has made extensive contributions in this field since 1949, has provided a comprehensive series of photographs of phase-contrast appearances of cells in his excellent monograph "Cytology of the blood and blood-forming organs" (1956) and references to the literature concerning phase-contrast microscopy of leucocytes are to be found in this work and in a recent article by Moeschlin (1957).

Although this method of examination has great advantages over more conventional techniques in the study of motility and the demonstration of nucleoli, mitochondria and cytoplasmic granules in normal and leukaemic leucocytes, and although it probably offers a more satisfactory means of distinguishing leukaemic precursor cells from one another and from lymphosarcoma cells than did previous methods, phase-contrast microscopy has not revealed any distinctive or characteristic feature in leukaemic cells. An illustrative series of photographs is reproduced in Plates XVII and XVIII.

Supravital staining

A number of stains can be used to demonstrate intracellular cytoplasmic structures in living leucocytes without causing the immediate death of the cells (Sabin, 1923; Cunningham and Tompkins, 1930; Hetherington, 1936). The stains commonly used for this purpose include neutral red, which colours vacuoles, Janus green and pinacyanol, which specifically stain mitochondria, and certain basic dyes such as brilliant cresyl blue and Nile blue which stain both vacuoles and mitochondria rather less specifically. The combination of dyes chosen, usually neutral red and either Janus green or pinacyanol, is made up in varying proportions in absolute alcohol, spread on very clean glass slides and allowed to dry so that a uniform layer of dye covers the slide surface. The need for varying proportions of the dyes arises from the toxicity of the mitochondrial stains; too much readily kills the cell, while staining does not occur at all until an almost lethal concentration is present. A drop of blood placed on a clean coverslip is inverted on each of a series of prepared slides, the coverslip edges are sealed with paraffin and the cells examined after about twenty minutes, preferably on a warm stage. Full descriptions of techniques for the preparation and examination of specimens, and accounts of the appearances of leucocytes stained supravitally, have been given by Whitby and Hynes (1936), Cunningham and Tompkins (1938), Schwind (1950) and Bessis (1956).

The earlier workers, before the introduction of phase-contrast microscopy, regarded supravital staining as a major step forward, and gave detailed descriptions of supposedly characteristic arrangements of neutral red vacuoles and mitochondria, classifying and differentiating cells according to the numbers and distribution of these bodies. No specifically leukaemic features were described, but the method was thought to offer a clear distinction between myeloblasts, monoblasts and lymphoblasts. Hall (1938), from an extensive critical study, concluded that this and many other claims were unjustified, and although Schwind (1950) believed that the methods employed by Hall were technically unsatisfactory, he agreed that supravital staining was only rarely of value in differentiating primitive mononuclear cells. With the availability of phase-contrast microscopy for the study of cytoplasmic organoids, little use is now made of the supravital stains except as an occasional adjunct to phase-contrast examination.

Electron microscopy

The electron microscope, in which rays of electrons, controlled by a series of "condenser" "objective" and "ocular" electrostatic and magnetic lenses, traverse the object and form an image on a photographic plate or fluorescent screen, has a resolving power more than a hundred times that of the conventional light microscope. The system presents several special problems, however, when attempts are made to use it for the study of cell structure. Perhaps the most important of these arises from the rapid fall in velocity of the electrons as they penetrate the object, so that objects more than 0·2 μ in thickness prove impenetrable. A second major difficulty is the need for cells to be dried before they can be examined, since electrons fail to travel in straight lines unless in a vacuum. Thirdly, contrast in the final image is dependent upon density differences in neighbouring parts of the object, and in most cells these differences are small. Methods of accentuating small density differences, by varying the electron velocity or by metallic impregnation or shadowing of the object, have therefore been the subject of much study.

The ways in which these problems have been overcome, and the results of subsequent study of blood and bone-marrow cells, have been fully reviewed by Bessis (1956, 1957). Objects of suitable thickness can be obtained by encouraging the spontaneous tendency to spread thinly over a supporting surface manifested by some cells, by mechanical disruption of other cells with release of cytoplasmic constituents, by partial enzymatic digestion of cell contents, or, most satisfactorily, by techniques developed since 1953 for cutting sections between 0·02μ and 0·1μ in thickness (Porter and Blum, 1953; Kautz and Marsh, 1954; Pease, 1955).

Despite the great power of electron microscopy in demonstrating cellular constituents below the range of light microscopes, the results of its application in the field of leucocytic structure and leukaemia have hitherto been rather disappointing. In experimental leukaemia in AK mice, Dalton and associates (1950) and Gross, McCarty and Cohen (1952) described the presence of possible virus particles after destruction of the cell, and Bessis and Thiery (1955*a* and *b*), using the thin-section technique, found many cytoplasmic granules of uncertain nature. In cells from human leukaemias no such particles have been detected. Bessis (1957) claimed to have found certain microcrystals, perhaps related to the Auer bodies visible in ordinary microscopic preparations, but of a very much smaller size. He also noted crescentic clear zones, up to 1μ by 3μ in size, of filamentous or regularly granular constitution, but clearly distinguished from the denser surrounding cytoplasm. These formations were observed in 3 to 30 per cent of leukaemic myeloblasts and promyelocytes, but did not occur in later granulocytes or in cells of other series. Bessis thought these zones to be areas of cytoplasmic degeneration, perhaps related to the "degenerative zones" observed in some cells affected by virus (Koprowski, 1956). The existence of these areas in leukaemic myeloblasts has not yet been confirmed by other workers. Bessis (1957) has also recorded the observation of short rods 1 to 1·5 μ in length and about 0·2 μ across among the mitochondria in leukaemic lymphoblasts and lymphocytes subjected to crushing. They are unlike Auer bodies or myeloblastic microcrystals and appear to be formed during the process of grinding. Bessis observed similar bodies in a plasmacytoma, but never in normal cells or leukaemic myeloblasts or monoblasts. This observation also awaits confirmation. Further studies on submicroscopic degenerations in leukaemic cells and on the possible existence of virus-like particles in human as well as

mouse and fowl leukaemias are being actively pursued at present (Dmochowski and Grey, 1958).

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CHAPTER 6

THE LEUKAEMIC CELL

II. Biochemistry and Metabolism

The cytochemical techniques discussed in the last chapter provide valuable information on the intracellular localization of many cell constituents. The methods are not for the most part strictly quantitative and allow only rough comparisons between the differing chemical and enzymatic properties of normal and pathological cells. Quantitative studies of leucocyte biochemistry, carried out on separated cell populations, supplement the cytochemical data, but suffer from the disadvantages inherent in studies of large numbers of cells treated as a unit, namely, that the populations rarely consist of a single cell type without contamination with others, and differing activities among members of the same cell type in a given population cannot be appreciated. For each biochemical assay there are, of course, further problems: differences between the artificial *in vitro* test system and probable *in vivo* activity; inactivation of cell constituents during separation and extraction; uncertainties about pH optima for enzymatic processes *in vivo*; and so forth. Nevertheless, much information has already been gained from the application of these methods, and in the present chapter this information and its interpretation will be discussed.

The leukaemic process may be considered within the general context of all proliferative and neoplastic states, whose biochemistry has been actively studied in recent decades, and occasional reference to data obtained from the study of solid tumours may be relevant to parallel investigations in leukaemia. Excellent surveys of the wide field of cancer biochemistry are to be found in the monographs of Stern and Willheim (1943), Greenstein (1954) and Cowdry (1955). The ease with which samples of leucocytes can be obtained repeatedly without risk to the patient enables biochemical studies of these cells to be carried out in man, and the specimens obtained, although not made up of pure cell lines, can be simply quantitated by total and differential leucocyte counts, and the component cell types can be more completely separated by centrifugation and other means than is possible with the cells of most solid tumours. The leucocytes are therefore especially well suited to this form of biochemical investigation, and much work has been done on both leukaemic and non-leukaemic pathological states. Aspects of this work have been reviewed by Valentine (1956).

Protein and nucleoprotein metabolism

Gross changes in the general protein composition of leukaemic or neoplastic cells are scarcely to be expected, since grossly abnormal cells would be unlikely to survive and multiply. Nevertheless, there can be little doubt that changes in the character and rate of growth of leukaemic leucocytes are associated with alterations in the mechanisms of protein metabolism, involving perhaps different pathways of synthesis, different metabolic

precursors, altered enzymatic activity with changes in substrates and reaction products, and possibly small but important changes in the internal structure of the protein molecules (Rondoni, 1955; Haddow, 1955). Aspects of protein metabolism in leukaemia which have been extensively studied include the general patterns of plasma proteins in different forms of the disease, the distribution, functions and utilization of amino acids, the special importance of sulphydryl compounds, the activity of enzymes concerned with amino acid metabolism, alterations of DNA and RNA in leukaemic leucocytes, changes in folic acid and citrovorum factor activity, and problems of uric acid metabolism.

The plasma proteins in leukaemia. Extensive study of total protein levels in the plasma or serum, and search for anomalies in component fractions separated by electrophoresis, have revealed no consistent or characteristic changes in any form of leukaemia. Essentially similar findings have been reported by Brown and his associates (1948), Petermann, Karnofsky and Hogness (1948), Rundles, Coonrad and Arends (1954), Creyssel and his colleagues (1957) and many other workers.

In acute leukaemias the total amount of serum protein is normal or slightly diminished, while electrophoretic patterns show much variability, though of minor degree. The commonest abnormalities are reduction in the albumin fraction and increases in different globulin constituents, chiefly alpha$_1$ and alpha$_2$ globulins and gamma globulins. Fibrinogen levels in plasma remain usually within the normal range. These changes have generally been most marked in states of severe relapse and are probably non-specific responses to infection and hepatic involvement, although Franzini, Campanini and Perone (1955) found protein anomalies more common in monocytic than in other forms of acute leukaemia and believed that a specific predisposition towards dysproteinaemia might exist in this variety of the disease.

In chronic granulocytic leukaemia the patterns observed have closely resembled those of the acute leukaemias and the minor abnormalities appear to be attributable to the same non-specific factors, being most prominent in advanced states of disease, with intercurrent infections and probable infiltration of the liver.

The serum proteins in chronic lymphocytic leukaemia may show more striking variations from normal than are seen in other forms of leukaemia. This is to be expected in view of the close relationship of lymphocytes to plasma cells and other cellular components of the lymphoid and reticulo-endothelial system, with their probable functions in elaborating antibodies and other globulins. Nevertheless, no consistent abnormalities of electrophoretic protein patterns occur, the majority of patients with clinically mild chronic lymphocytic leukaemia showing no differences from normal. At later stages of the disease a marked increase in gamma globulins may be found, or there may be conspicuous hypogammaglobulinaemia. Figures given in the published reports quoted above differ widely as to the relative incidence of conspicuous gamma globulin abnormalities, but the discrepancies probably depend upon the proportion of patients in advanced or terminal stages studied by the different groups of observers, and it appears very likely that 50 per cent or more of patients in the later stages of the disease come to manifest significant changes in gamma globulin levels.

Further, much less common, protein abnormalities sometimes occurring in chronic lymphocytic leukaemia involve the production of atypical plasma globulins quite unlike those normally produced. They include globulins resembling those often found in multiple

and Poncher, 1952; Kelley and Waisman, 1957). An increase in glutamic acid, phenylalanine, tyrosine and isoleucine was found in the plasma of patients with acute leukaemia. Chronic myeloid and lymphatic leukaemias were associated with raised levels of plasma glutamic acid, phenylalanine, alanine and proline, but not of tyrosine or leucine. Diminished levels of asparagine and threonine were found in acute leukaemias, and a low mean level for arginine occurred in chronic myeloid leukaemia. All these differences were slight, though regarded by the authors as significant. In some cases they were increased and in others reduced during treatment.

There is little evidence of any consistent abnormality of urinary amino acid excretion in leukaemia. Nour-Eldin and Wilkinson (1955*b*) reported values within the normal range, and Waisman, Pastel and Poncher (1952) did not find the elevated plasma tyrosine levels in leukaemia to be reflected in increased urinary tyrosine excretion. Awapara (1957) has, however, detected β-aminoisobutyric acid in the urine of each of a series of leukaemic patients, whereas this amino acid is not commonly excreted in detectable amounts by normal subjects. There is evidence that β-aminoisobutyric acid may be the end-product of thymine catabolism, and its increased excretion in leukaemia could therefore result from a high rate of DNA catabolism. A sharp rise in urinary levels of this acid after antimitotic therapy, observed in several cases, lends support to this hypothesis.

Sulphydryl compounds. Chromatograms of lymphocytic, granulocytic and platelet amino acids regularly showed an inverse relationship between the quantities of glutathione and cysteine or cysteinylglycine (Rouser, 1957). Unfortunately the literature on leucocytic glutathione (GSH) is difficult to interpret and compare because different methods of assay appear to have given discrepant results. Contopoulos and Anderson (1950), using an iodometric method, found high concentrations of GSH in acute leukaemia and in both chronic lymphocytic and granulocytic leukaemia. The highest levels were found with the most immature cell populations and appeared to parallel the malignancy of the disease. In leucopenic and aplastic states levels of GSH were low. The changes in GSH concentration did not, however, show any clear relationship to the total leucocyte count. With successful specific therapy the raised GSH levels in leukaemia returned towards normal. Other investigators have usually found elevated GSH values in chronic granulocytic leukaemia, but there is less agreement over the levels present in acute leukaemias and chronic lymphocytic leukaemias (Platt, 1931; Weil, Aschkenasy and Capron, 1939; Bichel, 1946; Hardin *et al.*, 1954; Green and Martin 1955). Hardin and his associates, for example, using a nitroprusside assay method, found GSH levels normal or increased in chronic myelocytic leukaemia, normal or slightly decreased in acute leukaemias, and consistently below normal in chronic lymphocytic leukaemia. The levels were unaffected by therapy. Differences in method may perhaps explain the conflicting results.

In a series of papers Weisberger and his associates have drawn attention to the especially important role of the sulphydryl amino acid L-cysteine in the metabolism of leucocytes (Weisberger and Heinle, 1950; Weisberger and Levine, 1952, 1954*a* and *b*; Weisberger, Suhrland and Seifter, 1956; Weisberger, 1957). The severe leukopenia normally induced by nitrogen mustard is modified by previous administration of L-cysteine. Protection is not given by L-cysteine administered after the injection of nitrogen mustard, nor by previously administered D-cysteine, and the closely apposed sulphydryl, amino and car-

boxyl groups of L-cysteine appear to confer a structural specificity essential for the protective effect.

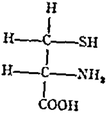

FIG. 8. L-Cysteine

Many compounds with structures related to L-cysteine have been tested for their ability to modify nitrogen-mustard-induced leukopenia, and removal, alteration, substitution or reorientation of any of the three reactive sulphydryl, amino and carboxyl groups results in loss of the protective action. Apart from L-cysteine, the only compounds found to exert a protective influence are homocysteine and glutathione.

Studies with S^{35}-labelled radioactive L-cystine showed that this amino acid was rapidly incorporated into leucocytes and that the leucocytes of acute leukaemia and of chronic myeloid leukaemia exhibited greatly increased avidity, taking up as much L-cystine in 20 minutes as normal leucocytes did in 48 hours. The cells of chronic lymphocytic leukaemia did not show an increased rate of cystine incorporation. Weisberger has pointed out (1957) that this rapid turnover of amino acid by immature leukaemic leucocytes is not limited to L-cystine and may be simply a manifestation of the general increase in metabolic activities characteristic of immature cells. Thus, Baker, Zamecnik and Stephenson (1957) demonstrated that white blood cells from patients with chronic myeloid leukaemia were able to incorporate significantly greater amounts of C^{14}-DL-leucine into proteins *in vitro* than did normal leucocytes. They regarded this finding as a reflection of cell immaturity and proliferation. Nevertheless, the protective action of L-cystine against the induction of leukopenia by nitrogen mustard warrants particular interest in its metabolism. Moreover, lack of cysteine or cystine in the diet leads to the development of leukopenia in experimental animals (Dinning, Payne and Day, 1950) and reduces the incidence of leukaemia (White, White and Mider, 1947), while granulocytes cultured *in vitro* on synthetic media deficient in either L-cysteine or L-cystine rapidly degenerate (Baldini and Sacchetti, 1953). The importance of this amino acid in leucocyte metabolism is further emphasized by the actions of selenium cystine, an analogue of cystine in which selenium replaces sulphur in the molecule, in decreasing the spleen size and leucocyte count in patients with leukaemia (Weisberger and Suhrland, 1956).

Enzymes concerned with amino acid metabolism. Nour-Eldin and Wilkinson (1955*a*) found high levels of glutamic acid in the cells from acute leukaemia, and Rouser (1957) also recorded variable increases in plasma glutamic and aspartic acids in acute and chronic lymphocytic leukaemia. Two of the enzymes concerned with glutamic acid metabolism, glutamic acid dehydrogenase and glutamic oxalacetic acid transaminase, have been studied manometrically by Waisman and associates (Waisman, Monder and Williams, 1956; Waisman, 1957). Cells from acute and chronic leukaemias of every cytological variety showed dehydrogenase activity about four times as high as normal [illegible] here

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although large numbers of units are probably involved in a chain-like union. Patterns for the molecular structure of nucleic acids have been proposed by Watson and Crick (1953*a* and *b*) and Feughelman *et al.* (1955) on experimental data summarized by Wilkins (1957). Both DNA and RNA contain chemically demonstrable 3′,5′-phosphodiester

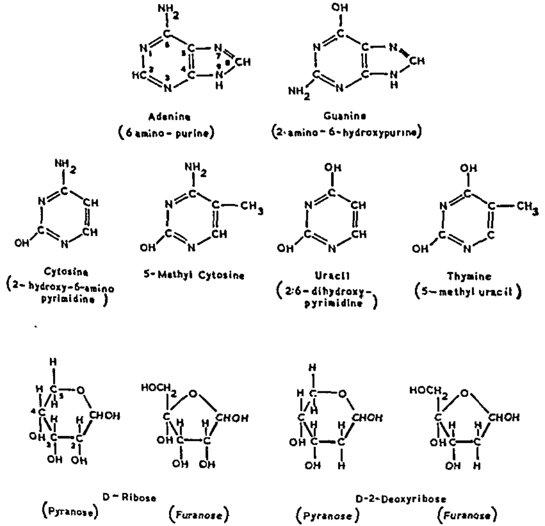


FIG. 9.

linkages, while physical studies have shown that nucleic acid molecules are long thread-like structures. A section of a single nucleotide chain may therefore be represented as in Fig. 10.

Further analysis of DNA has demonstrated that adenine and thymine occur in equal proportions, as do guanine and cytosine, suggesting that the bases occur in pairs, a

was considerable variation in enzyme activity within each group of patients. A similar increase was shown by the leucocytes of patients with cancer but not by the white cells in non-cancerous diseases. For any individual patient the glutamic acid dehydrogenase activity of the leucocytes did not fluctuate with changes in the total white cell count and was usually unaffected by therapy. Patients in complete haematological remission do not, however, appear to have been studied, and it is possible that the enzyme level may return to normal in such cases, since in one patient with chronic myeloid leukaemia described by Waisman (1957) there was a sharp fall in dehydrogenase activity at a time when substantial remission had been induced by oxapentamethylenediethylenethiophosphoramide, although earlier courses of myleran (busulphan), X-ray, and nitrogen mustard, which had been less effective clinically, produced no enzyme decrease. Estimations of glutamic oxalacetic transaminase in leucocytes and plasma from 29 cases of leukaemia of different types showed values within the normal range and the levels were unchanged by chemotherapy or X-irradiation.

The synthesis and nature of DNA and RNA in leukaemic leucocytes. The capacity of leukaemic cells to proliferate rapidly with the formation of much new protein, and the probability that at least some forms of leukaemia arise by genetic alteration or mutation from existing normal leucocyte precursors, draw attention particularly to nucleic acid content and metabolism in leukaemic leucocytes. A wealth of evidence leaves no doubt that both DNA and RNA are of major importance in protein synthesis and in cell division and hereditary transmission. Researches in this field have been widely reviewed by Brachet (1957). DNA is chiefly concerned with genetic mechanisms, whereas RNA plays an essential part in protein synthesis, and it would not be unreasonable to expect abnormalities of both DNA and RNA to be present in leukaemic cells. Extensive studies have therefore been made of the nucleic acids, their purine and pyrimidine bases, and the precursor substances and enzyme systems involved in their biosynthesis in normal and leukaemic leucocytes. The normal pathways of nucleic acid synthesis are unfortunately extremely complex, but since differences are known or suspected to exist in the uptake of various precursors in normal and leukaemic cells, and since many anti-leukaemic chemicals exert their effect by metabolic antagonism at points along the biosynthetic pathway, a brief description of the major steps at present believed to occur normally during the process of nucleic acid formation is a necessary preliminary to discussion of the experimental findings in leukaemia.

Nucleic acids are made up of purine and pyrimidine bases, a sugar, probably ribose or deoxyribose, and phosphoric acid. Both DNA and RNA contain the purine bases adenine and guanine. Both types of nucleic acid also contain the pyrimidine base cytosine and in addition DNA contains thymine and 5-methyl cytosine, while RNA contains uracil. The sugar component of RNA derived from a variety of tissues has been shown to be D-ribose, and that of DNA is almost certainly deoxyribose. The structural formulae of all these compounds are shown in Fig. 9. Condensation of purine or pyrimidine bases with ribose or deoxyribose results in the formation of the nucleosides adenosine, guanosine, cytidine, 5-methyl cytidine, uridine and thymidine, and phosphorylation of these nucleosides produces the nucleotides adenylic acid, guanylic acid, cytidylic acid, uridylic acid and so on.

The way in which nucleotides are built up to form nucleic acids is still uncertain,

protein synthesis and as templates determining the sequence of amino acids in the polypeptide chain. General references to the many aspects of nucleoprotein activity and metabolism may be found in The Biochemistry of the Nucleic Acids, by Davidson (1956),

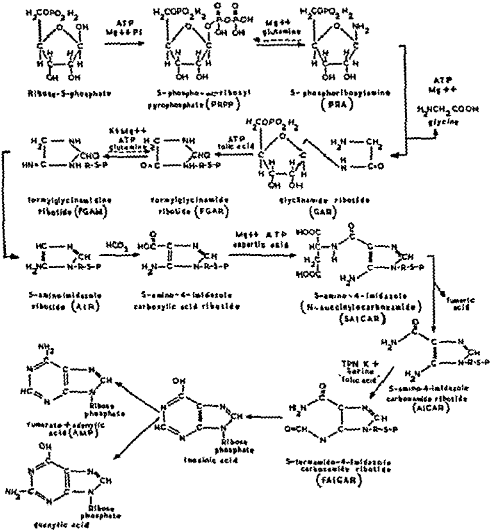


FIG. 12. Tentative scheme of purine nucleotide biosynthesis.

The Nucleic Acids, edited by Chargoff and Davidson (1955), and The Structure of Nucleic Acids and their Role in Protein Synthesis, edited by Crook (1957).

Enough has been said, however, to make it clear that comparative studies of normal and leukaemic cell nucleoprotein metabolism may be concerned with the *de novo* synthesis of purine and pyrimidine nucleotides, the utilization of exogenous bases, and the source

suggestion gaining additional support from experiments showing a high level of hydrogen bonding between bases within the molecule. The architectural models of Watson and Crick and Feughelman and his associates provide a general explanation for all these findings, by assuming the existence in the DNA molecule of two polynucleotide chains, helically coiled around a common axial core, and held together by hydrogen bonds between base pairs, a purine base in one chain being linked with a pyrimidine base in the other

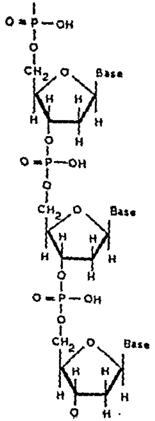


Fig. 10. 3′, 5′-*phosphodiester linkages in the nucleotide chain.*

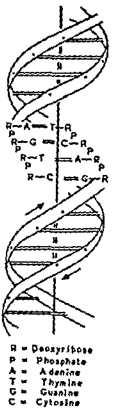
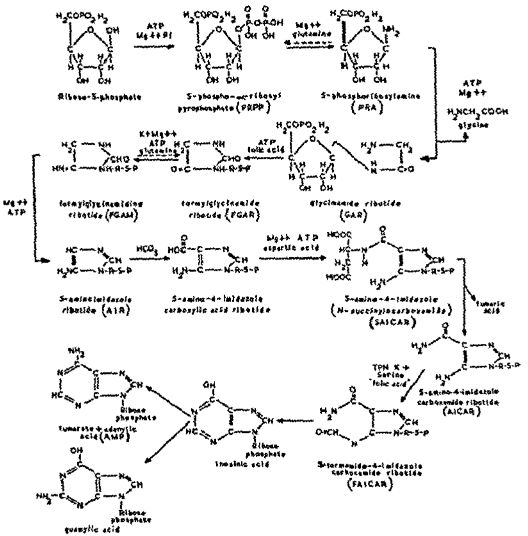


Fig. 11. Postulated structure of the DNA molecule.

(Fig. 11). X-ray diffraction and viscosity studies have given general support to this hypothesis.

The RNA molecule does not show evidence of base-pairing as in DNA and no reasonable molecular structure has yet been put forward. Moreover, the mode of combination of both DNA and RNA with proteins to form nucleoproteins remains uncertain, except in the case of deoxyribose nucleoprotamine, for which an elegant structure has been determined by Feughelman *et al.* (1955). The chemical details of nucleoprotein structure, both known and conjectural, are beyond the scope of this chapter, as are the theories concerning the mode of action of nucleic acids as energy sources for the formation of peptide links in

protein synthesis and as templates determining the sequence of amino acids in the polypeptide chain. General references to the many aspects of nucleoprotein activity and metabolism may be found in The Biochemistry of the Nucleic Acids, by Davidson (1956),



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of the ribose component, as well as the mechanism of linkage of these units into the polynucleotide chain and the final biosynthesis of nucleic acids and nucleoproteins.

Synthesis of purine and pyrimidine nucleotides *de novo*. All mammalian tissues studied are able to synthesize purine nucleotides from simple precursors, a capacity demonstrable *in vitro* with tissue slices and cell suspensions (Balis and Dancis, 1955; Le Page, 1953). Among the cells tested and shown to possess this ability are leucocytes (Hamilton, 1953). The multiple steps involved in the synthetic process are probably alike in most tissues and the results of numerous experimental investigations are incorporated in the tentative synthetic scheme given in Fig. 12.

The biosynthesis of pyrimidines has been less fully elucidated. A possible route of synthesis of the pyrimidine nucleotides uridine, thymidine and cytidine is shown in Fig. 13.

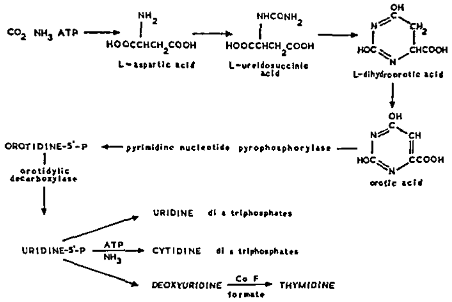

FIG. 13. Tentative scheme of pyrimidine nucleotide biosynthesis.

Utilization of exogenous bases. The biosynthetic processes outlined in Figs. 12 and 13 show that, according to the experimental evidence available at present, free purines and pyrimidines are not formed *in vivo*, the base ring structure being completed after the incorporation of phosphate and ribose. Nevertheless, most tissues are able to utilize supplies of exogenous bases by a hitherto unexplained mechanism which supplements or by-passes the native process of synthesis from small units. Brown and Roll (1955) have outlined the alternative pathways of nucleotide formation in the following, very simplified, form. (Fig. 14).

The scheme indicates that exogenously supplied adenine may be converted into polynucleotide guanine and vice versa, since these transformations are known to occur in bacterial metabolism and probably also take place in mammalian tissues.

The source of the ribose component. The steps by which glucose may be converted

to D-ribofuranose-5-phosphate have been established (Dickens, 1953; Bernstein, 1953; Horecker and Mehler, 1955). Two alternative pathways may be followed, one involving a series of transketolase–transaldolase reactions and the other a process of oxidation, decarboxylation and reduction starting from glucose-6-phosphate and 6-phosphogluconic acid. The individual steps in ribose-5-phosphate synthesis will not be further detailed here, since there is no evidence that they are altered in leukaemia and they are not subject to antimetabolic chemotherapeutic interference. Nevertheless, the need for ribose-5-phosphate from the earliest stages onwards in the synthesis of nucleotides provides an important link between the increased formation of nucleic acids and the changes in leucocyte glycolysis and respiration which occur in leukaemia. The possibility has been considered (Goldthwait, 1957) that increased conversion of glucose to ribose-5-phosphate may provide the primary driving force in nucleotide synthesis.

Formation of polynucleotide chains. The later stages in polynucleotide biosynthesis are imperfectly understood, and the relationship of DNA and RNA formation is uncertain.

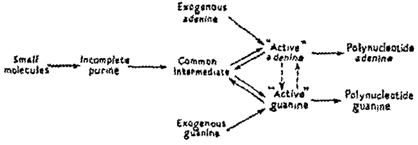

FIG. 14.

Reduction of ribose and methylation of uracil into thymine would enable a transformation from RNA to DNA to take place, but it is unlikely that such conversion plays any major part in DNA formation, and most recent experimental work supports the view that DNA and RNA are independently synthesized from low molecular weight precursors (Brachet, 1957).

Abnormalities of nucleoprotein metabolism in leukaemia

Quantitative studies on the content of DNA and RNA in leukaemic cells from blood and bone marrow as compared with normal cells have been carried out by several investigators. Metais and Mandel (1950) found no significant difference in content of DNA phosphorus (DNAP) between normal and leukaemic leucocytes. Davidson, Leslie and White (1951) confirmed this observation regarding DNAP and found that cell RNAP levels in leukaemic marrow were also not significantly different from those in normal marrow. The authors found this surprising in view of the rich cytoplasmic basophilia, removable by ribonuclease, in most primitive cells, but they noted that the mean RNAP per cell for peripheral-blood leucocytes was significantly lower than the corresponding mean for marrow cells both in normal and in the leukaemic series, and that leukaemic peripheral blood, with its increased proportion of immature cells, showed a significantly higher mean cell RNAP than was found in normal peripheral blood. Studies by other

of the ribose component, as well as the mechanism of linkage of these units into the polynucleotide chain and the final biosynthesis of nucleic acids and nucleoproteins.

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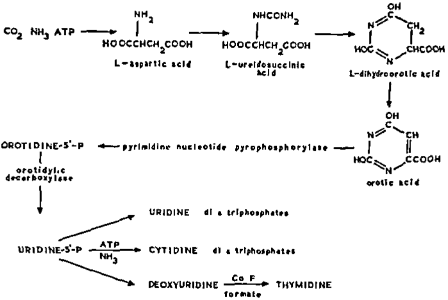

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The source of the ribose component. The steps by which glucose may be converted

Will, Glazer and Vilter (1957) discussed the possible role of nucleases and nuclease inhibitors in leukaemia. The DNAase content of leukaemic cells was not different from normal, but an inhibitor of DNAase was found to be deficient in the cells of acute leukaemia and chronic lymphatic leukaemia and less markedly deficient in chronic myeloid leukaemia. This deficiency of DNAase was not found in leukaemoid reactions, leucocytoses or myeloid metaplasia. The authors therefore argued that the relatively uninhibited activity of DNAase in leukaemia might be responsible for the increased capacity for division manifested by leukaemic cells, since it is possible, though not biochemically certain, that DNAase may catalyse polymerization as well as depolymerization of DNA. The DNAase inhibitor studied by Vilter and his associates is not, however, the only DNAase inhibitor known to exist. A separate inhibitor investigated by Kurnick *et al.* (1953) was present in increased amounts in leukaemia. This contradictory behaviour of inhibitor concentrations could be reconciled with the leukaemic proliferation if the first inhibited the postulated polymerization activity of DNAase and the second the depolymerization activity, but such a concept is purely speculative at present.

Neoplastic and leukaemic cells possess marked capacities for *de novo* synthesis of nucleotides, and Balis, Van Praag and Brown (1955) have shown that many tumours incorporate exogenously supplied preformed purines to a much smaller extent than do normal intestine, liver, kidney or spleen. Rapidly proliferating normal tissues such as regenerating liver and bone marrow apparently show a similar preference for the *de novo* pathway (Brown and Balis, 1957), so that this characteristic cannot be regarded as peculiar to neoplastic states. The reluctance of tumours to utilize preformed purines applies particularly to guanine (Bennett *et al.*, 1955; Balis, Van Praag and Brown, 1955), and the difference from normal in this respect is very conspicuous. Skipper (1957) has presented evidence that neoplastic cells may be rich in guanine deaminase, which might rapidly break down guanine before incorporation was possible. Whether these biochemical features exist also in rapidly proliferating but non-leukaemic primitive leucocytes is not known.

Folic acid and citrovorum factor activity in leukaemia

The folic acid–citrovorum factor system is so closely involved in nucleoprotein synthesis (see Fig. 12, p. 105) that alterations might be expected to occur in leukaemia, and the clinical response to folic acid antagonists in acute leukaemia suggests that the leukaemic cell is, at least for a time, highly sensitive to deprivation of citrovorum factor (CF). Swendseid, Bethell and Bird (1951) found the mean cell levels of CF to be raised in leukaemia, particularly in acute forms of the disease with many immature cells in the blood. Ellison and Hutchinson (1957) reported data in general agreement with these findings, an increase in apparent CF activity being present in leucocytes from cases of acute leukaemia and some terminal cases of chronic leukaemia. The increase in activity could not be strictly correlated with the percentage of blast cells in the peripheral blood and the abnormality was presumably present in mature as well as immature cells. The microbiological method used for CF estimation in these experiments, however, measured also thymidine and perhaps other unidentified substances, and at least part of the apparent CF activity was due to the presence of thymidine released by leucocyte autolysis.

Studies of serum levels and urinary excretion of CF have shown no significant difference

workers using various methods of nucleic acid determination have given roughly comparable results (Menten and Willms, 1953; Will, Glazer and Vilter, 1957; Loeb, Wright and Hall, 1957). The quantity of DNA per cell has been found to be normal or slightly elevated in acute leukaemia and normal in chronic leukaemias. The small excess of DNA sometimes found in acute leukaemia is probably due to chromosomal polyploidy, and haploid leukaemic cells do not differ from haploid normal cells in DNA content. The cell content of RNA is increased in acute leukaemia and in chronic myeloid leukaemia, but not apparently in chronic lymphatic leukaemia, and the RNA level falls in response to successful treatment.

Unfortunately, the range of cell types present invariably in normal and often in leukaemic specimens of blood and bone marrow is wide, and figures for mean cell nucleic acid concentrations are relatively uninformative. It seems extremely likely that these biochemical estimates have done no more than confirm the cytological and cytochemical observations that primitive cells are rich in cytoplasmic and nucleolar RNA, that leukaemia is associated with an increase in primitive cells, most marked in the acute forms of the disease, and that the precursor cells are reduced in numbers towards normal by appropriate therapy. The leukaemic myeloblast and promyelocyte is being compared with the normal granulocytic and erythroblastic cell series in the marrow or with the mature polymorphonuclear cells and lymphocytes of the normal peripheral blood. Such comparisons are of very doubtful value; the leukaemic myeloblast should be compared with the normal myeloblast, a much more difficult matter until studies can be performed on isolated cells or uniform cell populations rather than on mixed cell systems.

More valuable information may be derived from studies of the nucleotide constitution of DNA and RNA in leukaemia, since preliminary analyses suggest the existence of differences in the relative content of purine and pyrimidine bases in the nucleic acids of leukaemic as compared with normal cells. Polli and Semenza (1955) found the viscosity and molecular weight of DNA obtained from leukaemic blood to be abnormal, and Gavosto and Pileri (1956) noted an abnormal uracil content in leukaemic RNA. Will, Glazer and Vilter (1957) have also observed increases in pyrimidine and purine bases in acute leukaemia, with changes in relative proportions of uracil and thymine, marked increase in cytosine and only slight increase in guanine. To what extent these changes are due to the high RNA·DNA ratio of primitive cells is uncertain, and once again it must be emphasized that the normal control findings, with which the figures for leukaemic cells are compared, have been derived from mixed normal blood and marrow populations and not from normal precursor cells. However, it is unlikely that the structure of nucleic acids alters at the same time as the quantitative relationships of RNA and DNA during normal cell maturation, and mixed cell populations are therefore a little more satisfactory as control material for qualitative studies of nucleic acid structure than for studies of RNA: DNA ratios. This difficulty of providing realistic controls applies also to the promising method of chromatographic nucleotide separation employed by Willoughby and Waisman (1957). Chromatograms of acid-soluble extracts of whole blood and separated blood components from normal and leukaemic human donors disclosed differences in nucleotide content of both quantitative and qualitative nature, but the abnormal fractions, of uncertain identity, found in leukaemic blood may be characteristic of immaturity rather than leukaemia.

was not necessarily correlated with the number of circulating leucocytes, and was part of a general increase in nucleic acid metabolism. The increase was not found in chronic lymphatic leukaemia, perhaps because breakdown of lymphocytes may be followed by reutilization of large DNA fragments in fresh lymphocyte nucleoprotein synthesis, as suggested by the isotope experiments of Hamilton (1957). Leukaemic patients in remission had normal serum and urinary uric acid levels. During treatment with folic acid antagonists a fall in uric acid occurred, presumably from interruption of purine synthesis, whereas purine analogue antimetabolites caused a rise in uric acid, because the site at which they interfere with nucleic acid synthesis is after purine ring formation and the unused purines accumulating in these circumstances may be converted to uric acid although their utilization for nucleic acid formation has been blocked. The alkylating agents, radiation and adrenal corticosteroids also produce a rise in uric acid, probably due to destruction of cells and breakdown of nucleic acids rather than to interruption of synthesis. These concepts can be represented diagrammatically as in Fig. 15 (after Krakoff, 1957).

An interesting gradation in uric acid levels among a variety of patients with myeloproliferative diseases has been reported by Hickling (1958). The highest blood uric acid levels were very closely associated with the presence of large numbers of megakaryocytes in the bone marrow and spleen, and were accordingly most often found in megakaryocytic myelosis, myelofibrosis, and atypical cases of polycythaemia or chronic myeloid leukaemia with unusual megakaryocytic hypertrophy. The mechanism of excessive uric acid production in these cases is uncertain.

Glycolytic and respiratory metabolism in leukaemia

The great interest in this field of metabolism, manifested in many publications during the past twenty years, derived principally from the observations and hypotheses of Warburg concerning the existence of a typically "malignant" energy-producing pattern of activity in tumour cells (Warburg, 1930). This pattern involved high aerobic and anaerobic glycolysis with defective respiration. In a recent expansion and restatement of his hypothesis, Warburg (1956) put forward the view that cancer development resulted from an irreversible destruction of respiration which might lead to the death of some cells but was followed in those cells that survived by the replacement of respiration energy by fermentation energy. Chronic intermittent oxygen deficiency due to circulatory disturbances or respiratory poisons might therefore be expected to be carcinogenic, and there is certainly some experimental evidence that this may be the case. The general validity of Warburg's theory has been examined and criticized in detail in a recent discussion by Weinhouse (1955), who concluded that oxidative metabolism in tumours was not in fact impaired, and that the high aerobic glycolysis of cancer cells was an independent phenomenon, not resulting from a lesion of cell respiration.

Work on glycolysis and respiration in normal and leukaemic leucocytes has been reviewed by Beck and Valentine (1953) and Valentine (1956), who have stressed the difficulties of obtaining normal counterparts to leukaemic precursor cells and the inability to separate individual cell types satisfactorily from mixed cell populations. Despite these drawbacks, there appears to be general agreement that both normal and leukaemic leucocytes have predominantly an aerobic glycolytic metabolism, and that, although oxygen consumption, glucose utilization and lactic acid production are less in cell homogenates

between leukaemic and non-leukaemic patients (Hutchinson and Burchenal, 1954), although there is some evidence that when folic acid is given as a test dose leukaemic patients convert more of it to CF and excrete less as unaltered folic acid than do normal individuals (Swendseid *et al.*, 1952; Girdwood, 1953).

Uric acid in leukaemia and related diseases

Raised serum and urinary uric acid levels have often been observed in patients with leukaemias, and the levels are sometimes further increased during treatment. This may

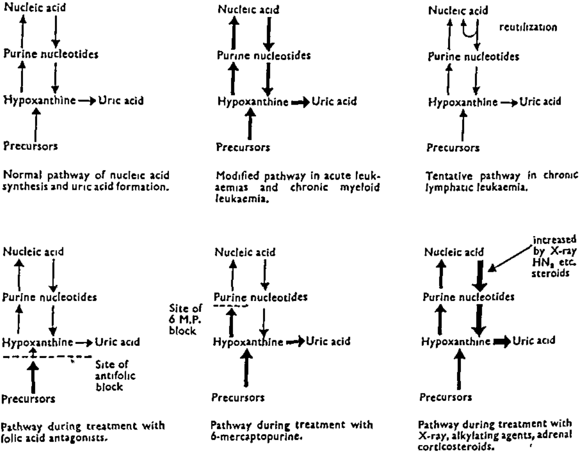

Fig. 15. Uric acid metabolism in leukaemia.

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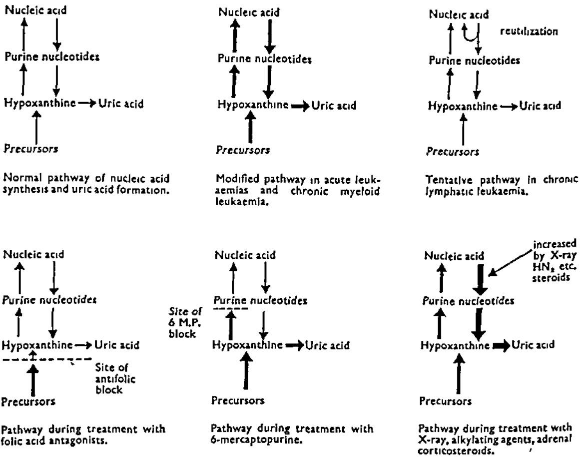

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b) demonstrated that hexokinase acted as the chief rate-limiting enzyme in both normal and leukaemic leucocyte homogenates. The lower glycolytic rate in leukaemic cells could be correlated with a deficiency in hexokinase and an associated diminution in adenosine diphosphate (ADP) production. Investigation of the phosphogluconate pathway showed that a higher percentage of metabolized glucose followed this route in leukaemic than in normal leucocytes, a difference possibly attributable to hexokinase deficiency.

A high level of serum phosphohexose isomerase has been shown to exist in chronic granulocytic leukaemia but not in lymphocytic leukaemia (Israels and Delory, 1956). The level of activity parallels the rise and fall in the total granulocyte count, and Israels and his associates (1958) suggest that the enzyme is liberated from disintegrating granulocytes. The low isomerase content of lymphocytes and their relatively long life-span would account for the failure to find an increase in serum isomerase in chronic lymphocytic leukaemia.

While the investigations of Beck have revealed a decreased cellular lactic dehydrogenase (LDH) level in leukaemia, studies by other workers have disclosed elevated levels of the enzyme in the serum of leukaemic patients. Bierman and his associates (1957) found that 84 of 91 patients with leukaemia had serum LDH values above the normal adult range. Serial studies in 7 of these patients with leukaemias of different kinds showed serum LDH values fluctuating in parallel with the clinical state. Comparable findings were reported by West, Heller and Zimmerman (1958), but they found high LDH levels only in granulocytic and acute leukaemias and not in chronic lymphocytic leukaemia. Hill and Jordan (1957) observed a similar increase in serum LDH activity in AKR mice inoculated with a transplantable lymphatic leukaemia. When the transplant gave rise to generalized and ultimately fatal disease, the LDH level continued to rise, most markedly just before death. If the transplant took but subsequently regressed, an initial rise in serum LDH was followed by a gradual return to normal. An increase in serum LDH activity is not a certain indication of leukaemia or neoplasia; but there may well be a relationship between LDH activity and growth. In this connection it is of note that the normal levels in children are substantially higher than those in adults.

Estimations of leucocytic glycogen

Cytochemical studies of the glycogen content in blood cells have been supplemented by a number of quantitative biochemical studies, confirming the existence of this substance in blood and marrow leucocytes, particularly those of the later granulocytic series, and emphasizing changes encountered in leukaemia and leukaemoid reactions (Wagner, 1946, 1947; Valentine, Follette and Lawrence, 1953). Valentine and his associates found an average value of 75 mgm. glycogen per 10^{10} granulocytes, with a range of 47 to 119 mgm., in normal human subjects. Changes were not observed in association with fasting or feeding, nor in diabetes, nor after adrenal cortico-steroids. In infective states with polymorphonuclear leucocytosis there was an increase in mean levels, and a still greater increase was found in polycythaemia with leukaemoid features, where an average value in 15 patients was as high as 116 mgm. In chronic myeloid leukaemia on the other hand, the low mean glycogen level of 38 mgm. per 10^{10} cells was found in a study of 14 patients. Morphologically similar peripheral-blood pictures in leukaemia and leukaemoid reactions were still associated respectively with low and high granulocyte glycogen values,

from chronic leukaemias than in normal leucocytes, there is no suggestion of a sharp and specifically "malignant" change in carbohydrate metabolism in leukaemic leucocytes.

Nevertheless, certain differences between leukaemic and normal cell glycolytic metabolism have been shown to exist. Beck (1955) studied the activity of the glycolytic

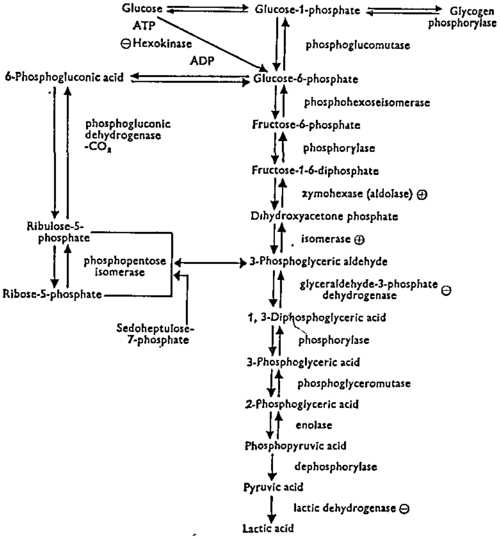

FIG. 16. The Meyerhof-Embden glycolytic system and the hexose-monophosphate shunt.
⊕ Enzymes increased in leukaemic cells.
⊖ Enzymes decreased in leukaemic cells.

enzymes required in the Meyerhof–Embden scheme (Fig. 16) in normal and leukaemic cells. All the necessary enzymes were present in both cell types, but leukaemic cells contained less glyceraldehyde-3-phosphate dehydrogenase and lactic dehydrogenase, and more aldolase and isomerase activity than did normal cells. In further studies Beck (1958*a* and

b) demonstrated that hexokinase acted as the chief rate-limiting enzyme in both normal and leukaemic leucocyte homogenates. The lower glycolytic rate in leukaemic cells could be correlated with a deficiency in hexokinase and an associated diminution in adenosine diphosphate (ADP) production. Investigation of the phosphogluconate pathway showed that a higher percentage of metabolized glucose followed this route in leukaemic than in normal leucocytes, a difference possibly attributable to hexokinase deficiency.

A high level of serum phosphohexose isomerase has been shown to exist in chronic granulocytic leukaemia but not in lymphocytic leukaemia (Israels and Delory, 1956). The level of activity parallels the rise and fall in the total granulocyte count, and Israels and his associates (1958) suggest that the enzyme is liberated from disintegrating granulocytes. The low isomerase content of lymphocytes and their relatively long life-span would account for the failure to find an increase in serum isomerase in chronic lymphocytic leukaemia.

While the investigations of Beck have revealed a decreased cellular lactic dehydrogenase (LDH) level in leukaemia, studies by other workers have disclosed elevated levels of the enzyme in the serum of leukaemic patients. Bierman and his associates (1957) found that 84 of 91 patients with leukaemia had serum LDH values above the normal adult range. Serial studies in 7 of these patients with leukaemias of different kinds showed serum LDH values fluctuating in parallel with the clinical state. Comparable findings were reported by West, Heller and Zimmerman (1958), but they found high LDH levels only in granulocytic and acute leukaemias and not in chronic lymphocytic leukaemia. Hill and Jordan (1957) observed a similar increase in serum LDH activity in AKR mice inoculated with a transplantable lymphatic leukaemia. When the transplant gave rise to generalized and ultimately fatal disease, the LDH level continued to rise, most markedly just before death. If the transplant took but subsequently regressed, an initial rise in serum LDH was followed by a gradual return to normal. An increase in serum LDH activity is not a certain indication of leukaemia or neoplasia; but there may well be a relationship between LDH activity and growth. In this connection it is of note that the normal levels in children are substantially higher than those in adults.

Estimations of leucocytic glycogen

Cytochemical studies of the glycogen content in blood cells have been supplemented by a number of quantitative biochemical studies, confirming the existence of this substance in blood and marrow leucocytes, particularly those of the later granulocytic series, and emphasizing changes encountered in leukaemia and leukaemoid reactions (Wagner, 1946, 1947; Valentine, Follette and Lawrence, 1953). Valentine and his associates found an average value of 75 mgm. glycogen per 10^{10} granulocytes, with a range of 47 to 119 mgm., in normal human subjects. Changes were not observed in association with fasting or feeding, nor in diabetes, nor after adrenal cortico-steroids. In infective states with polymorphonuclear leucocytosis there was an increase in mean levels, and a still greater increase was found in polycythaemia with leukaemoid features, where an average value in 15 patients was as high as 116 mgm. In chronic myeloid leukaemia on the other hand, the low mean glycogen level of 38 mgm. per 10^{10} cells was found in a study of 14 patients. Morphologically similar peripheral-blood pictures in leukaemia and leukaemoid reactions were still associated respectively with low and high granulocyte glycogen values,

presumably indicative of metabolic differences in reserve energy stores, and perhaps biochemically related to the parallel differences in alkaline phosphatase content.

Quantitative biochemical studies of acid and alkaline phosphatases

The cytochemical studies of leucocytic phosphatases reviewed earlier demonstrated important changes in concentration of the alkaline enzyme in leukaemic leucocytes. Biochemical measurements on separated white blood cell populations have provided confirmation of these changes. The work of Haight and Rossiter (1950), Valentine and Beck (1951), Beck and Valentine (1951), Valentine *et al.* (1952), Moloney and Lange (1954), Wiltshaw and Moloney (1955) and others has made it clear that alkaline phosphatase activity in the white cells of leukaemic patients is greatly reduced, whereas in neutrophilic leucocytes of infection and in non-leukaemic myeloproliferative states there is a sharp increase. Valentine and his co-workers (1957) have shown also that ACTH and adrenal corticosteroids given over a 72-hour period produce a roughly threefold increase in unit leucocyte alkaline phosphatase in most normal subjects. In pathological states with an initially high enzyme level some further increase may occur, but in chronic myelocytic leukaemia the response to steroid administration is nearly always slight or absent, phosphatase levels remaining in the low leukaemic range. In the majority of cases of chronic myeloid leukaemia there is no biochemically demonstrable change in the low phosphatase levels during successful therapy with either chemical agents or radiation; and the high phosphatase of other myeloproliferative states is similarly uninfluenced by treatment, even when the white cell numbers return to normal (Valentine *et al.*, 1957; Kenny and Moloney, 1957). As we have seen, small but significant changes may be detected cyto-chemically.

While this general picture of consistent differences, not grossly influenced by therapy, between the leucocytic alkaline phosphatase levels in leukaemic and leukaemoid reactions has been confirmed histochemically and biochemically in the great majority of patients studied, both Valentine and his associates and Kenny and Moloney have drawn attention to occasional paradoxical findings. In a small proportion of cases alkaline phosphatase activity has been within the leukaemic range, whereas histological autopsy findings indicated myeloid metaplasia, and a few examples of high phosphatase figures have been encountered in histologically probable myeloid leukaemias. These equivocal cases may represent transitional stages between the myeloproliferative states. Valentine and others (1957) have also reported two examples of return to normal in leucocytic phosphatase activity during full remission in chronic myeloid leukaemia. The capacity to respond to adrenal corticosteroids was recovered during these metabolic remissions, the cells being indistinguishable from normal in their alkaline phosphatase content and response. These and other equivocal findings are further discussed in Chapter 16.

Less interesting results have accrued from acid phosphatase studies. The activity of this enzyme in the cells from neutrophilic leucocytoses of infectious origin is not appreciably altered, and there appears to be little significant change in the leukaemias or in other myeloproliferative states with leukaemoid features (Valentine, 1956).

Biochemical studies of other enzymatic constituents of leukaemic cells

Beta-glucuronidase. An increase in the activity of this enzyme, which is able to hydrolyse and perhaps synthesize glucuronide conjugates, has been observed in many

non-leukaemic neoplastic tissues (Anylan and Fishman, 1947), but an increase does not occur in leukaemia. Anylan, Gamble and Hoster (1950) and Follette, Valentine and Lawrence (1952) reported studies of beta-glucuronidase in various forms of leukaemia, but their results were not in full agreement. However, lymphocytes possessed less enzyme than granulocytes, whether leukaemic or not, and values in both acute and chronic leukaemias were either within the normal range or depressed.

Esterases and lipases. These enzymes, concerned with the hydrolysis of short- or long-chain fatty acid esters, are present in normal leucocytes (Rossiter and Wong, 1949; Nachlas and Seligman, 1949). Their concentration in the leucocytes from a variety of pathological states has been studied by Hardin and others (1955), who found values within the normal range in most cases of leukaemia, whether acute or chronic, lymphocytic or myelocytic, although high values were observed in two cases of monocytic leukaemia. Both esterase and lipase activity appear to be diffusely distributed among leucocytes of different kinds and no clear-cut variations in disease have been established.

Other enzymes. Leucocytes are known to possess many more enzymes, including nucleases, trypsin, amylase, adenosinase and cathepsin (Barnes, 1940), catalase (Stern, 1932) and peptidase (Stern *et al.*, 1951), but no significant variations in the concentration of these enzymes in leukaemic cells have yet been observed.

Histamine in leukaemia

The presence of histamine in suspensions of leucocytes, predominantly in the granulocyte fraction, has long been recognized (Code, 1937*a* and *b*), and very high values have often been observed in chronic myeloid leukaemia (Thiersch, 1947; Valentine, Pearce and Lawrence, 1950). The studies of Riley (1953) and Ehrich (1953) suggested that the basophil cell might be the chief site of leucocytic histamine, and Valentine and his associates (1955) have reported a very close correlation between blood histamine levels in chronic myeloid leukaemia and the absolute numbers of basophils present. The high basophil percentage in many cases of chronic myeloid leukaemia would therefore explain the increased histamine activity, without postulating any leukaemic change in cell histamine carriage. The absence of histamine increase in most leukaemoid reactions and in neutrophilic leucocytosis, and the occasional high figure in polycythaemia, are also readily explicable in the light of this basophil-histamine relationship.

Changes in trace elements in leukaemia

Gibson and his associates (1950) found the zinc content of leucocytes in chronic myeloid leukaemia greatly reduced below comparable figures for normal cells, and Underwood (1956) noted that a similar reduction occurred in chronic lymphocytic and monocytic leukaemia. Injections of stable zinc gluconate failed to raise the levels or to influence the course of the disease, but when clinical and haematological remission occurred under appropriate therapy the leucocytic zinc content rose to normal. Koch, Smith and McNeely (1957) reported data on copper and zinc levels in plasma, and copper, zinc, iron, magnesium, molybdenum, lead, nickel, cadmium, tin and chromium in tissues from patients with lymphomatous diseases, including acute and chronic leukaemias. Elevated plasma-copper levels were found in most cases, and these could be correlated with increases in alpha$_2$ and alpha$_3$ globulin, suggesting that the hypercupremia was due to a raised coeruloplasmin

level rather than to an increase in copper ions. The plasma-zinc levels were also raised, the increase being parallel to that of copper. Tissue levels of copper, zinc and iron showed a wide range in material from control subjects without cancerous or lymphomatous disorders, and in leukaemia no consistent changes were found in the levels in liver, spleen, lymph glands or in a variety of other tissues although variations were wider than normal. Leucocytes were not separately analysed. No significant alterations in tissue content of the other trace elements were found.

Plasma and white cell ascorbic-acid levels in leukaemia and allied states

Bodansky, Wroblewski and Markardt (1952) compared plasma and white cell ascorbic-acid levels in groups of normal subjects, patients with cancer and patients with chronic non-cancerous diseases. In both the latter groups decreased values were found in cells and plasma. Waldo and Zipf (1955) carried out similar studies on 42 patients with leukaemias of various kinds and 30 patients with lymphosarcoma, reticulum cell sarcoma, Hodgkin's disease or multiple myeloma. All these patients had low values for ascorbic acid concentrations in both plasma and white cells, and the mean values for the group were much lower than the corresponding mean values for 50 normal subjects. During treatment with corticosteroid hormones the ascorbic acid levels fell still further, and even when full remissions were induced by this therapy the levels did not return towards normal. When remissions were induced by radiation or chemotherapy, however, a rise in ascorbic acid concentration occurred.

The increased demand for ascorbic acid appears to be a general phenomenon in many chronic diseases as well as cancer, although the evidence for increased utilization is most convincing in neoplastic disorders (Minor and Ramirez, 1942). The low vitamin level in plasma and leucocytes probably reflects a depletion of total body stores, but the mechanism of this depletion is not clear, and it certainly does not appear to be a specifically leukaemic abnormality.

Vitamin B_{12} concentrations in serum and leucocytes in leukaemia

Beard, Pitney and Sanneman (1954) observed a greatly increased concentration of bound vitamin B_{12} in the serum of patients with chronic myeloid leukaemia, and a similar increase was reported in acute myeloblastic and monocytic leukaemia by Beard, Pitney, Sanneman, Sakol and Moorhead (1954). Studies by Mollin and Ross (1955) on material from 56 patients with leukaemias of various kinds confirmed the existence of high serum levels of vitamin B_{12} in subacute and chronic myeloid leukaemia, but revealed values within the normal range in patients with acute leukaemia whose marrows showed little or no differentiation beyond the myeloblast or lymphoblast stage. Normal levels were also found in chronic lymphocytic leukaemia and in multiple myelomatosis. In a further group of patients with polycythaemia vera, myelosclerosis, or chronic non-leukaemic leucocytosis some increases in serum concentration of the vitamin were observed, but normal values were found in agranulocytosis and aplastic states. General confirmation of these findings has been reported by Rachmilewitz *et al.* (1957).

Mollin and Ross pointed out that increased B_{12} concentrations were in all cases associated with active granulocytic proliferation, and they noted that levels were observed to

fall when proliferation became less intense during successful treatment of chronic myeloid leukaemia and polycythaemia. They therefore argued that the high serum levels of B_{12} might be brought about by the release of bound vitamin from disintegrating granulocytes in the peripheral blood and tissues. Normal leucocytes have been shown to contain significant amounts of B_{12} (Harris, 1952), and leukaemic leucocytes of whatever kind do not apparently differ appreciably from normal in B_{12} content or binding power (Thomas and Anderson, 1956). Since the vitamin content is much the same in lymphocytes and granulocytes, the marked difference in serum levels in chronic lymphocytic and granulocytic leukaemias would suggest a much greater rate of white cell disintegration in the latter disease, if the serum concentration of B_{12} is to be attributed to vitamin release from the breakdown of leucocytes. This explanation may well be correct, since estimates of leukaemic cell life-span in recent years have tended to give figures far greater for lymphocytes than for granulocytes, so that the rate of granulocyte turnover may be between 5 and 20 times that of the lymphocyte (Osgood *et al.*, 1954; Ottesen, 1954; Osgood and Krippaehne, 1955). The changes in serum vitamin B_{12} concentration in granulocytic leukaemia therefore probably reflect the rapid cell turnover in that disease and do not represent a fundamental biochemical abnormality.

Lipid estimations in leukaemia

Although a macroscopically noticeable fatty turbidity is often to be seen in the plasma of leukaemic patients, surprisingly few quantitative studies of plasma or leucocytic lipids in leukaemia have been made. Pernokis and Freeland (1941) found the total lipids and fatty acids of whole blood, serum and plasma to be much increased above normal in all of nine patients with chronic myeloid leukaemia and three with chronic lymphocytic leukaemia. Parallel estimations of cholesterol, calcium, phosphorus and non-protein nitrogen gave normal values. Cytochemically, leukaemic cells do not manifest obvious increases in lipid stainable with Sudan black B, but in view of the demonstration by Costello *et al.* (1947) that the uptake of P^{32} in the phospholipids of squamous cell carcinoma in experimental animals was more than twice that of normal epidermis, and the finding of Williams *et al.* (1945) that the total lipid content of butter-yellow-induced hepatomas was twice that of normal liver, further work on cell and plasma lipids in leukaemia might prove informative.

General Comment

Despite the rapidly increasing understanding of fundamental processes of cell metabolism and the immense amount of experimental work on the biochemical activities of leukaemic cells, no clear evidence of metabolic disorder peculiar to leukaemia or of probable importance in leukaemogenesis has yet emerged. The nearest approach to a consistent and significant change is the low alkaline phosphatase activity in the granulocytes in myelogenous leukaemias, but the function and importance of this enzyme remains uncertain. Most of the other abnormalities described in this chapter are reasonable concomitants of rapid cellular proliferation or are of questionable significance in view of the heterogeneity of the cell populations studied. Nevertheless, it would seem to be only a matter of time before a clear knowledge is obtained whether leukaemic cell metabolism is basically abnormal or not, and perhaps continued study of DNA structure is most likely

level rather than to an increase in copper ions. The plasma-zinc levels were also raised, the increase being parallel to that of copper. Tissue levels of copper, zinc and iron showed a wide range in material from control subjects without cancerous or lymphomatous disorders, and in leukaemia no consistent changes were found in the levels in liver, spleen, lymph glands or in a variety of other tissues although variations were wider than normal. Leucocytes were not separately analysed. No significant alterations in tissue content of the other trace elements were found.

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to provide this information, since the DNA molecule is presumably responsible for transfer of any existing leukaemic abnormality from one generation of cells to the next.

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CHAPTER 7

THE LEUKAEMIC CELL

III. Antigenicity and Immunology

IMMUNOLOGICAL methods provide a highly sensitive means for distinguishing differences in the chemical composition of large antigenic molecules. Proteins which appear similar under ordinary chemical investigation can sometimes be readily differentiated by analysis of specific antigen-antibody reactions. Although the problem may be relatively simple when single antigens are compared, it becomes increasingly difficult when the material to be analysed consists of complex antigenic mixtures such as exist in cells and plasma. The search for new or altered antigens in the leucocytes and plasma of patients with leukaemia has therefore been delayed until recent years by the absence of suitable techniques, and is at present being pursued in only a small number of laboratories. Nevertheless, the potentialities of the immunological method in the study of cell alterations in disease are sufficient to warrant close consideration of the results so far achieved.

The study of leucocyte antigens is beset with technical difficulties. Leucocytes are extremely difficult to separate from other formed elements of the blood, particularly red cells, and procedures employed for separation are inclined to cause cell damage of uncertain extent. Lyophilized leucocytic emulsions have sometimes been used, but these are usually derived from populations of mixed cell type and reactions confined to a particular variety of leucocyte cannot therefore be detected. A diversity of techniques for the demonstration of leucocyte antigen-antibody reactions has, however, been devised. Methods in current use, all regrettably open to criticism, have been reviewed by Dausset (1956, 1957). The simplest and most widely used procedure is agglutination *in vitro*, a method long employed in experimental studies of the action of strong anti-leucocytic hetero-agglutinins, as reviewed by Cajano and Maurea (1950). By this means Moeschlin and Wagner (1952) demonstrated the presence of leucocyte agglutinins in the serum of patients with pyramidon-induced agranulocytosis. Agglutinating antibodies in the serum of certain leucopenic patients were similarly detected by Dausset and Nenna (1952) and Goudsmit and van Loghem (1953) and subsequent confirmation has been provided by several observers (Martensson and Vikbladh, 1954; Miescher, 1954; Ruggieri *et al.*, 1954; Muller, 1956; Brittingham, 1957). The interpretation of leucocyte clumping is not always straightforward, however, especially in the presence of infection, pregnancy, haemorrhage or any other stimulus to accelerated leucopoiesis, when the phenomenon of "leukergy" develops, with appearance in the circulation of leucocytes having an increased stickiness and tendency to agglomerate (Fleck, 1952). Lysis and phagocytosis of leucocytes in the presence of anti-leucocytic serum have been used to observe and assess the extent of antigen-antibody reactions (Finch, Ross and Ebaugh, 1953; Miescher, 1953; Robineaux, 1954; Bessis, 1955). Sensitization of leucocytes by antibodies has also been demonstrated

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concentrations employed were cytotoxic. Steinberg and Martin (1944, 1945, 1946) succeeded in obtaining satisfactory leuco-agglutination by using a critical concentration of leucocytes in rabbit antileucocytic sera, dilutions being made with normal saline and the reaction speeded by centrifugation. Antisera were prepared against normal and leukaemic leucocytes of various kinds and agglutination and absorption experiments carried out. The authors concluded that mature and immature lymphocytes and granulocytes each possessed specific antigenic substances, reflecting different chemical components in each type of cell. No evidence of antigen specificity peculiar to leukaemic cells was found. The complement fixation reaction was used by Maculla (1947) to compare the antigenic structure of various normal and tumour tissues from the mouse. Tumour cells were found to possess antigens in common with normal cells, but to have additional components in some cases. Specific anti-leukaemic cell antibodies were not, however, detected. An antibody or group of antibodies specific for the cells of an induced leukaemia in DBA/2 mice was obtained by Thompson (1955). Antisera prepared in rabbits gave complement fixing reactions with both normal and leukaemic lymphocytic antigens, but absorption with normal lymphocytic antigen left an antibody residue reacting only with leukaemic tissue. Despite the *in vitro* activity, anti-leukaemic sera did not afford protection to other DBA/2 mice against transplanted tumour growth. The effect of heterologous immune sera prepared against neoplastic cells, when injected *in vivo* into animals with transplanted tumours, has usually been insignificant, weak or non-specific, and difficult to ascribe to an antigen-antibody reaction peculiar to the neoplastic cell. In the case of certain mouse lymphomas, however, definite inhibition of transplants by immune sera has been observed (Nettleship, 1945; Nungester and Fisher, 1954). A careful study of the effects of 43 immune sera prepared in rabbits with antigens consisting of non-lymphocytic mouse tissue, non-neoplastic lymphocytic tissues from thymus and lymph nodes, and cells of seven different transplantable lymphomas originating in C3H, AKR and Strong A mice, showed that all the sera killed the lymphoma cells *in vitro* in the presence of complement (Mohos and Kidd, 1957). The sera varied in potency, however, those against lymphoma cells being most active and those against non-lymphocytic tissues least active. Absorption experiments indicated the existence of antibodies corresponding to specific antigens of lymphocytes, whether normal or neoplastic. Immune sera containing this antibody component inhibited lymphoma development *in vivo*, whereas immune sera against non-lymphocytic tissues had no protective effect. The specific antigens appeared to be shared by normal and neoplastic lymphocytes, but there was some evidence of further inherent differences in the types of lymphoma cell studied, since antisera were sometimes more active against their parent cell strain than against normal lymphocytes or lymphoma cells of different strains.

Direct experimental studies with immune sera have therefore not yet clearly demonstrated the existence of specifically leukaemic antigens in the leucocytes of leukaemia, although some evidence in favour of their probable existence has been found. In this respect the position resembles that in the general field of neoplasia, where attempts to demonstrate specific antigens in tumours not caused by viruses have yielded few positive results (Hauschka, 1952; Zilber, 1957).

There is, however, considerable indirect evidence of an abnormal immunological state in leukaemia, since the plasma in many cases manifests activity of an "auto-immune"

by the use of an antiglobulin test (Steffen and Schindler, 1955; Van Loghem, Van der Hart and Borstel, 1957), and by complement fixation (Milgrom *et al.*, 1957), while specific precipitation of leucocytic antigens by appropriate antisera has been carried out, using modifications of the ring-test and two-dimensional gel methods of Oudin (1946) and Ouchterlony (1948), by Seligman, Grabar and Bernard (1954, 1955). Seligmann (1957) has used also an immuno-electrophoretic technique for separating precipitation reactions involving different leucocytic antigens.

By the use of this multiplicity of technical methods the antigenic structure of leucocytes has been increasingly revealed in recent years. At least ten specific nuclear or cytoplasmic antigens have been distinguished in normal leucocytes by precipitation in gel (Seligmann, Grabar and Bernard, 1955). The possibility that leucocytes might be separable into groups with differing antigenic constitution, similar to the erythrocyte subdivisions, has been explored. Moeschlin and Schmid (1954) examined the reaction of leucocytes in sera from blood samples which were either compatible or incompatible with the blood of the leucocyte donor with respect to erythrocyte ABO grouping. In 174 incompatible cross-matches, 60 showed leucocyte agglutination, while in 206 compatible cross-matches only 10 showed leucocyte agglutination. Further studies by Dausset (1954), Maupin *et al.* (1955), Riis (1955) and Whyte and Yee (1956) have been taken to confirm the presence of antigens A and B on leucocytes from blood of the respective erythrocyte groups. Bakemeier and Swisher (1957) have pointed out, however, that techniques employing leucocyte suspensions with an unavoidable admixture of erythrocytes may give rise to false leuco-agglutination as a result of adherence of leucocytes to sensitized or damaged red cells. Leucocyte agglutinates consist chiefly of potentially phagocytic granulocytes, while lymphocytes usually fail to form clumps, and this observation supports their argument that erythrophagocytic activity is frequently the cause of apparent agglutination, particularly in the presence of thermolabile serum components and potentially haemolytic antibodies. Until homogeneous leucocyte suspensions, devoid of erythrocyte contamination, can be produced, the agglutination studies interpreted as demonstrating the presence of A and B antigens on leucocytes must be regarded with reserve.

Apart from the possible existence of A and B antigens absorbed onto or forming part of the surface of the leucocytes, specific leucocytic surface antigens distinct from those of erythrocytes have been shown to exist. These surface antigens appear to be of great importance in stimulating iso-antibody formation in patients receiving multiple transfusions, and immunization produced in this way is a major cause of febrile transfusion reactions (Dausset, 1954; Payne, 1957*a* and *b*). The specific leucocytic antigens are probably genetically transmitted and identical patterns appear to exist in monozygotic twins (Dausset and Brecy, 1957). A start has already been made on the separation of leucocyte antigenic groups, and Dausset *et al.*, (1957) have reported the presence of an antigen "Mac" in about 60 per cent of blood samples taken at random in France.

It is upon this background of rapidly growing knowledge of normal leucocyte antigenicity that studies of leukaemic cell antigens must be viewed. The earliest work was with experimentally produced antisera against human and animal leucocytes (Ledingham and Bedson, 1915; Lindstrom, 1927; Hueper and Russell, 1932; Chew, Stephens and Lawrence, 1936; Chew and Lawrence, 1937), but the antisera, while sometimes producing cell destruction *in vivo*, did not give good agglutination of leucocytes, perhaps because the

eluted from sensitized red cells derived from patients with chronic lymphocytic leukaemia. He found no evidence of antigen-antibody relationship between these two materials. Extracts of leukaemic leucocytes did, however, contain a potent haemolysin and agglutinin active against normal and trypsinized erythrocytes. This substance was found to differ from coating antibody eluted from the red cells of patients with acquired haemolytic anaemia (Pirofsky, 1957). In view of the demonstration by Leroy and Spurrier (1955) that beta-glucuronidase might be important in causing red cell lysis, and the possible agglutinating activity of anti-H substance (Morgan and Watkins, 1948), Pirofsky carried out experiments to determine whether the leukaemic cell extract contained either of these materials. The results excluded both. Haemolytic substances have previously been extracted from normal and neoplastic tissues (Weil, 1907; Maegraith, Findley and Martin, 1943; Gross, 1949) and a lysin-inhibitor complex derived from mouse tumour tissue by Ponder and Nesmith (1952) had some resemblance to Pirofsky's leukaemic cell extract. The materials were not, however, identical. Normal serum contained an inhibitor to the leukaemic cell extract which completely prevented haemolysis *in vitro*, and the *in vivo* activity of the lysin remains at present speculative. Agglutination was less strongly inhibited, and even slight agglutination may predispose to splenic sequestration and destruction of erythrocytes, so that the substance extractable from leukaemic leucocytes may contribute significantly to the increased rate of haemolysis in leukaemia.

Pirofsky (1956) argued that the demonstration in leukaemic cells of an agglutinating and lytic material reacting directly with erythrocytes would eliminate the need to explain red cell antibody-coating on a basis of auto-immunity. Positive antiglobulin tests could result from alteration of the red cell envelope, as in the experiments of Muirhead, Groves and Bryan (1954), who induced positive direct antiglobulin reactions by phenylhydrazine injections. The subject is far from settled and many discrepancies exist. Actively haemolytic material appears to be present in leucocytes from different varieties of leukaemia and is not confined to leukaemic lymphocytes; it is present also in the cells of some patients who manifest no obvious haemolytic process. On the other hand, clinical and laboratory evidence of haemolysis may be found without red cell sensitization. Probably more than one mechanism exists for the production of haemolytic anaemia in leukaemia, and both abnormal globulin synthesis by disordered lymphoid tissue and materials within the leukaemic cell active directly in erythrocyte agglutination and lysis contribute their respective influences to shortening the red cell life.

For the present, studies of apparent immunological abnormalities in leukaemia have failed to demonstrate with clarity any specifically leukaemic antigen within the leucocytes. Nevertheless, the strong suspicion remains that leukaemic cells might be found to differ immunologically from their normal counterparts if appropriately sensitive methods could be brought to bear. Reactions with normal guinea-pig serum provide an example of a difference between leukaemic and non-leukaemic leucocytes that has not yet been fully explored. Schwartz, Schoolman and Spurrier (1955) found that human leukaemic cells were almost always agglutinated by guinea-pig serum, only 6 of 58 cases failing to show the reaction, whereas only 5 of 369 cases with other diseases gave positive reactions. The significance and mechanism of this agglutination remain to be assessed.

A further immunological difference between leukaemic and normal cells has been described by Seligmann, Grabar and Bernard (1955). These authors used the method of

kind against erythrocytes and sometimes against leucocytes. Reference has been made earlier to reports of circulating leuco-agglutinins in cases of leucopenia of diverse aetiology, and these case reports include some of leukaemia (André, Dreyfus and Bessis, 1954; Bond and Rohn, 1955). Antibody activity showed some relationship to general activity of the disease, but was by no means uniformly present. Kissmeyer-Nielsen, Bichel and Bjerre-Hansen (1954) transfused blood from non-leukaemic leucopenic patients to subjects with normal blood pictures and found a marked transient leucopenic response; no such response followed transfusion of leukaemic leucopenic blood, suggesting that an antibody was not present in these cases, or, if present, was not active against normal leucocytes.

Evidence of erythrocyte sensitization by abnormal "auto-immune" gamma globulins in lymphocytic leukaemia has frequently been reported; the phenomenon is substantially confined to lymphocytic forms of the disease and is rarely, if ever, encountered in leukaemias of other cell types (Davis, 1944; Stats, Rosenthal and Wasserman, 1947; Rosenthal *et al.*, 1955). *Haemolytic anaemia is not uncommon as a complication of lymphocytic* leukaemia; Seaman and his associates (1957) found it present in 52 of 212 cases. The laboratory features of the disease are similar to those of idiopathic acquired haemolytic anaemia, with spherocytosis, reticulocytosis, bilirubinaemia, shortened erythrocyte life-span and positive direct and sometimes indirect anti-globulin test. The cytology of blood and marrow preparations shows a dual picture of lymphocytic proliferation and erythropoietic response to haemolysis.

Similar acquired haemolytic anaemias with red cell sensitization sometimes occur in association with lymphosarcoma and with non-malignant lymphocytic or reticulo-endothelial hyperplasias. The existence of abnormal lymphocytes in all these conditions has inevitably suggested that they may be responsible in some way for the development of the erythrocyte-coating globulins. This possibility has been discussed fully by Rosenthal *et al.* (1955), who concluded that the cytological and architectural disruption of lymphoid tissue in malignant lymphocytic disease might well have a functional counterpart with respect to the antibody-producing activity of the cellular components of lymphoid tissue, resulting in the elaboration of abnormal gamma globulins immunologically active against erythrocytes. The precise site of antibody formation is still uncertain, although the weight of evidence suggests plasmacytes, reticulo-endothelial cells or lymphocyte precursors rather than mature lymphocytes, but all these cellular elements are disturbed in malignant lymphocytic disease, and a functional disorder of immune globulin formation may certainly exist. Agglutinins active against red cells could, therefore, conceivably be formed without a specific antigenic stimulus, but alternative explanations for their formation must be considered. In the first place the postulated disorder of the antibody-forming system may involve a failure to recognize red cell antigens as native, so that they may excite an immune response as if they were foreign. There is little definite evidence in support of this hypothesis, since the antibodies are not specific for any particular red cell antigenic system, but it remains, nevertheless, a possibility. The lymphocyte itself has been considered a possible source of antigen. Rosenthal *et al.* (1955) discussed this question and concluded that the leukaemic or sarcomatous lymphocyte might be sufficiently far removed from normal in chemical composition to elicit a poorly specific antibody response. Pirofsky (1956) used precipitation and antibody neutralization techniques in the search for a component in leukaemic leucocyte extracts capable of reacting with antibodies

or multiple myelomatosis, and is reinforced by the finding of positive antiglobulin tests, using a sensitive technique, in 43 per cent of 451 cancer patients, whereas only 15 per cent of 241 control cases gave positive results (Green, Wakefield and Littlewood, 1957).

The hypothesis has points of attraction in offering an explanation for many phenomena of malignant disease and of leukaemia, but it is, of course, true that auto-immune erythrocyte sensitization and haemolysis are not uncommon in benign lymphoid hyperplastic conditions, such as glandular fever. Moreover, plasma cell increase is a striking feature of many malignant states, particularly when metastases exist, so that antibody globulin in these diseases may well be derived from orthodox sources rather than from tumour tissue. The intensive studies at present being conducted with recently devised techniques for antigenic analysis of leukaemic and normal leucocytes may be expected soon to provide definite evidence upon which the validity of these hypotheses, at least in the field of leukaemia, can be more firmly assessed.

The immunological data reviewed in this chapter present a thoroughly confused picture. Normal leucocytes have a complex but uncertain antigenic structure; leukaemic cells may have additional or different antigens or may lack some important antigens normally present. The leukaemic cell may perhaps be sufficiently foreign in structure to stimulate antibody formation, and may even itself elaborate globulins with antibody activity against constituents of erythrocytes or other cells. There has been a wealth of speculation on the role of immunological factors in the pathogenesis and development of leukaemia and malignant processes in general, but experimental evidence is still lacking on too many fundamental issues for any firm conclusions to be drawn.

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precipitation in gel to effect an immuno-chemical comparison between leukaemic and normal leucocyte extracts. They failed to find evidence of specific antigens peculiar to leukaemic cells in any of the major cytological varieties of leukaemia, but primitive cells from acute leukaemias were shown to lack one of the principal antigenic components of normal leucocyte extracts. This deficiency might, of course, be a manifestation of immaturity, since comparison with normal blast cells has been impossible, but the conception of leukaemic leukoblasts as cells devoid of some protein or enzymatic constituent normally present in leucocytes would accord well with the current "protein deletion" hypothesis of carcinogenesis postulated by Miller and Miller (1953), and the immunological concept of cancer elaborated by Green (1954, 1957, 1958). It is therefore appropriate to give some brief consideration to these hypotheses in the present context.

The protein deletion hypothesis arose from observations that metabolites of carcinogenic amino-azo dyes combined with proteins in affected tissues *in vivo* (Miller and Miller, 1952, 1953). The combination took place only in tissues susceptible to eventual carcinogenesis induced by the chemical agent used; species and tissues in which tumour formation could not be induced failed to bind the hydrocarbon. Further evidence suggested that protein–dye combination was an assential preliminary to later carcinogenesis, and that the tumour cells finally produced were deficient in those proteins which initially combined with the dye (Sorof and Cohen, 1951). An hypothesis was therefore put forward that combination of certain proteins with carcinogenic chemicals might inhibit further synthesis of the proteins and lead to their partial or complete deletion from daughter cells. A similar deletion of proteins and enzymes might be brought about by physical or viral agencies. The later development of tumours in tissues so affected would be a consequence of deletion of proteins or enzymes having essential activities in regulating cell growth and development, but this supposition so far lacks experimental support.

According to Green's immunological concept of carcinogenesis, modification of cell proteins by combination with azo-dyes or by other means produces a "foreign" substance within the cell, capable of exciting an immune response with the production of homologous antibodies. These antibodies may succeed in destroying the affected cells while still in a precancerous state, but continuous hyperplasia may take place with increasing immune reaction leading eventually to a cell adaptation involving the loss of "identity proteins". These "identity" or "marker" proteins are thought to enable scavenger cells to recognize displaced normal tissue cells, and to remove them without antibody formation being stimulated (Burnet and Fenner, 1949; Green, 1954). The loss of "identity proteins" in whole or in part produces a "neutral", freely invading, neoplastic cell, the degree of loss being correlated with the malignancy of the neoplastic state.

Green further argued (Green, Wakefield and Littlewood, 1957), that the final state of antigenic loss postulated to exist in the developed neoplastic cell would enable the cell to react immunologically with antibody formation to the challenge of the specific tissue antigens it had lost. Malignant growths derived from tissues normally capable of antibody production, in particular tumours of the reticulo-endothelial, lymphoid and plasma cell systems, might be expected to display this activity most markedly, but an immune response might be elicited also from malignant cells of other origins. The theory draws some support from the frequent existence of an auto-immune haemolytic state, with red cell sensitization, in patients with lymphocytic leukaemia, malignant lymphoid tumours,

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in vitro into neutrophil, eosinophil, and basophil myelocytes, which could subsequently form polymorphonuclear leucocytes. Cells of the lymphocytic series were incapable of granulopoiesis. Timofejewsky (1928) drew particular attention to his observation that the myeloblasts of acute leukaemia, though apparently unable to differentiate *in vivo*, could do so *in vitro*, and suggested that leukaemia should not be regarded as a malignant neoplasm, since the cells had not entirely lost their capacity to mature normally.

Hirschfeld (1927) was less successful in obtaining long-lived cultures in plasma clots, but was able to confirm the rapid degeneration of mature cells and the transformation of more primitive ones of each leucocytic series into spindle-shaped cells and sometimes into macrophages. He observed proliferation of myeloid elements but not their maturation. In this work and in later studies of lymphocytic leukaemic cell culture (Hirschfeld and Klee-Rawidowicz, 1928), the lymphocytes showed little sign of transformation, and the macrophages and fibroblast-like cells were considered to arise from monocytes or immature cells.

Many similar attempts to grow normal or leukaemic haemopoietic tissue in solid media, usually human or animal coagulated plasma, sometimes with the addition of Ringer's solution or embryo extracts, gave very comparable results (Veratti, 1928; Silberberg and Voit, 1931; Pierce, 1932; Wallbach, 1936), and in no case was a persistent growth of recognizable haemopoietic cells obtained. Fibroblast-like cells and phagocytic macrophages almost invariably came to dominate the cultures within a few days, but proliferation of granulocytes was frequently noted. On the question of maturation and differentiation of precursor cells, whether leukaemic or not, opinions were divided, some observers believing that limited maturation of myeloid elements took place and others that it did not. Conclusions had, of course, to be inferred from changes in relative proportions of cells in successive sections of material cultured for varying periods of time, since direct observation of cells during division and possible maturation was very difficult, and it was impossible to estimate the absolute numbers of the various cell stages present.

In contrast to these unsuccessful attempts to obtain persistent growth of human leukaemic cells on solid media were the results of Bichel (1938, 1939, 1940, 1952) with cells from mouse leukaemia. Fragments of leukaemic tissue were implanted in a medium containing three parts of rabbit plasma to one of fowl plasma, with a trace of chick-embryo extract to aid coagulation. Chick-embryo fibroblasts were added to the cultures since the leukotic cells were found to grow best in symbiosis with fibroblasts. The hanging-drop method of culture was used, and the cell fragments were reimplanted in fresh medium every three or four days, when the old substrate had begun to liquefy. After a number of successful transfers, the surviving cells grew strongly in a symbiotic manner, with leukaemic cells distributed widely between the fibroblast processes. Maintained cultures, lasting several months, were derived from a stem cell leukaemia, a chloromatous acute myeloid leukaemia and a plasma cell leukaemia. There was some irregular tendency towards maturation during the earlier stages of growth, but after some months all the cultures remained predominantly primitive in cytology, and upon inoculation into experimental animals each of the three cell types reproduced the corresponding form of leukaemia.

Successful maintenance of malignant lymphoblasts from a transplantable mouse lymphosarcoma has also been reported (De Bruyn, Korteweg and Van Waveren, 1949;

CHAPTER 8

THE LEUKAEMIC CELL

IV. *In vitro* Culture Studies

THE successful maintenance of living cells in artificial culture media outside the body, under conditions of controlled environment, would provide a most valuable instrument for fundamental research on cell characteristics. In the case of leukaemic cells it might answer the vexed question whether the basic abnormality is integral to the cell or imposed by the environment, and enable biochemical studies to be performed on uniform cell strains under simplified conditions of nutrition and respiration. Unfortunately, haemopoietic cells have proved very difficult to grow *in vitro*, and when surviving and proliferating cell strains have been obtained the resemblance to their natural counterparts in the body has usually been remote.

The first attempts to grow bone-marrow cells in artificial culture were reported by Carrel and Burrows (1910), who placed marrow fragments in hanging drops of clotted plasma, and observed the outgrowth of spindle-shaped cells after 3 days' incubation. The general technique of growth in plasma clots either as hanging drops or in Petri dishes or Carrel flasks was widely used by early investigators, chiefly with the object of elucidating the process of haemopoiesis. The methods and results were reviewed by Bloom (1938). In general, mature cells migrated out of the explant within a few hours and soon died, while immature cells persisted longer without very clear evidence of maturation but showing occasional mitotic figures, only to disappear in turn after 6 to 8 days. Within a few days most cultures contained predominantly long, spindle-shaped cells, often called fibroblasts, or large irregular non-granular mononuclear cells, usually regarded as monocytes or macrophages.

Awrorow and Timofejewsky (1914) used the plasma clot method to study *in vitro* the behaviour of leucocytes from the peripheral blood of patients with granulocytic leukaemia. Stained paraffin sections were made from cultures after varying intervals of incubation. Emigration of polymorphs and a few myelocytes was observed, while less mature cells were thought to be transformed into "polyblasts" or macrophages, with increased amount of cytoplasm, often vacuolated and containing phagocytosed material. Fibroblast-like cells were also prominent, and these were considered to arise from myeloblasts. Transformation of myeloblasts to myelocytes was not established, but some evidence of a myelocyte to metamyelocyte differentiation was noted. From many further experiments, using the same basic technique of leucocyte implantation in plasma clots, Timofejewsky and Benewolenskaja (1927, 1929) concluded that macrophages and fibroblast-like cells could arise from the lymphocytes and lymphoblasts of chronic lymphocytic leukaemia, or from haemocytoblasts or myeloblasts of acute and chronic granulocytic leukaemia, but that the primitive nucleolated cells of acute myeloblastic leukaemia were also able to differentiate

Kieler and Kieler (1954) used a somewhat different technique to obtain proliferation of mouse leukaemic cells *in vitro*. They set up cultures of fibroblasts from embryo mouse heart on coverslips in roller tubes, and when satisfactory growth had taken place they removed the central part of the original explant and replaced it with fragments of leukaemic mouse spleen. The spleen cells multiplied within the capsule of fibroblasts, and although cells migrating into the capsule tended to lose their identity and degenerate, continued growth of leukaemic cells was achieved by transferring the spleen explant to a new fibroblastic capsule each week. After 3 months the cytology of the leukaemic cells remained uniform and inoculation into susceptible animals produced death from leukaemia. The authors used this method in a study of the effects of amethopterin on sensitive and resistant cells from mouse leukaemias. The drug acted principally as a mitotic inhibitor, but all phases of mitosis appeared to be equally affected, and the specific arrest at metaphase observed in fibroblast cultures exposed to folic acid antagonists by Hughes (1950) and Jacobson (1952) was not seen. Significant inhibition of division was found at concentrations of 0·01 mgm. amethopterin per ml. of fluid medium surrounding sensitive cultures, whereas similar inhibition of resistant cultures required concentrations of 1 mgm. per ml. Cytoplasmic damage was observed only at very high concentrations and occurred then to an equal extent in both sensitive and resistant cells. Exposure to amethopterin in high doses for a week did not prevent either sensitive or resistant cells from producing leukaemia when transferred back to mice. The experiments did not provide evidence of amethopterin inactivation by resistant cells, since the drug concentration in the medium, measured by bacterial inhibition, was unaltered throughout.

Despite the success of these several techniques in providing established strains of leukaemic or lymphosarcomatous cells from mice, the satisfactory growth of human material *in vitro* has continued to provide intractable problems. Fieschi and Astaldi (1946) attempted to grow normal and pathological bone-marrow cells from man in a solid medium consisting of coagulated human or chicken plasma with embryo extract, surrounded by a liquid phase of human serum and Tyrode's solution. By renewing the liquid phase at intervals of 3 or 4 days, fresh nutrient could be provided and products of cell breakdown removed. There was initially active proliferation of myeloid elements and evidence of incomplete and atypical maturation of undifferentiated cells from normal marrow, with formation of normoblasts or granulocytes, but within about 10 days fibroblast-like cells had come to dominate the cultures and recognizable haemopoietic cells were few. The behaviour of marrow cells from patients with chronic myeloid leukaemia was very similar to that of normal marrow cells, with proliferation of myeloid cells and some limited and irregular maturation for a few days, followed by overgrowth of fibroblasts. Cultures of marrow from acute stem cell leukaemia showed a different picture, the primitive cells disappearing after 5 to 6 days and being replaced by histiocyte cells rather than fibroblasts. Fieschi, Cambiaggi and Sacchetti (1954) again confirmed the difference in behaviour between the cells of acute leukaemias and those of chronic leukaemias, even when the latter were in an acute, terminal, phase. When cells of acute leukaemia were cultured *in vitro* their survival was longer than that of chronic leukaemia cells, relatively few fibroblasts appeared, and even as late as 16 days after the cultures were set up cells were clearly surviving with "blastic" characteristics. The authors concluded that there must exist a clear genetic or metabolic difference between acute leukaemic cells and analogous cells

De Bruyn, 1949; De Bruyn and Gey, 1952). The technique employed was rather elaborate. Small pieces of tumour were explanted into roller tubes, in clots containing two parts of chicken plasma, one part of 50 per cent mouse-embryo extract in Tyrode's solution, and one part of 50 per cent chick-embryo extract in Tyrode's solution. The fluid medium surrounding the solid phase consisted of three parts of Tyrode's solution to one part of rat serum, since mouse serum proved to be toxic for mouse cells in culture. After 24 hours many lymphoblastic cells had migrated into the liquid phase and these were sucked off, centrifuged, and resuspended in fresh fluid medium. Single lymphoblasts were isolated from a small sample of the suspension by successive dilution in drops of cell-free medium on a mica coverslip at room temperature, using a micromanipulator and microscopic observation during the process. When the desired isolation of a single lymphoblast in a droplet on the coverslip had been achieved, a culture of mouse-embryo fibroblasts was superimposed upon it and the whole fixed in position by a drop of coagulated chicken plasma and embryo extract. After 2 or 3 days' growth on the coverslip, cultures were transferred to roller tubes and maintained for long periods. Subsequent observation revealed the presence in most cultures of three cell types; round lymphoblast-like cells, larger than lymphocytes, containing a large nucleus with a few nucleoli, devoid of peroxidase activity or phagocytic power and having rapid motility with the characteristic "hand-mirror" appearance shown by lymphocytes and lymphoblasts during movement, due to the nucleus being situated anteriorly and a short tail of cytoplasm trailing posteriorly; mesenchymal fibroblastic cells; and large phagocytic macrophages which were not always abundant and usually disappeared within a short time. Intraperitoneal or subcutaneous inoculation of susceptible mice with small numbers of isolated lymphoblast-like cells readily produced fatal lymphosarcoma, but actively growing mesenchymal cells failed to induce tumour formation when injected.

Prolonged survival of the cultures took place, and after more than 2 years actively proliferating subcultures were still available for study. At this stage some cultures were composed of mixed fibroblasts and lymphoblasts, growing in symbiosis, and capable of inducing lymphosarcoma in inoculated mice. Others were made up of fibroblastic mesenchymal cells only, and these were unable to give rise to tumours in injected animals. A third group of cultures contained lymphoblasts only, the fibroblastic elements having disappeared, and for several months after the emergence of this apparently pure strain of malignant cells, transmission to experimental animals was still followed by tumour growth, though with increasing latent periods. A gradual change in cell morphology occurred, however, with the appearance of large, sometimes binucleated, cells, and others much smaller than the original lymphoblasts, resembling mature lymphocytes. After 4 or 5 months these cells proved incapable of provoking tumour formation and they remained unable to do so even after subculture with added fibroblasts and embryo extract.

These experiments of De Bruyn were not, of course, with strictly leukaemic cells, but with the cells of a mouse lymphosarcoma, and it is particularly interesting that she has, according to De Brion (1956), succeeded in obtaining a pure, maintained, culture of myeloblasts derived from a myeloblastic leukaemia of the mouse, and capable of survival and proliferation in the absence of mesenchymal support. The cells preserved their morphological appearance and remained able to give rise to leukaemic tumours in inoculated animals.

total and differential cell counts performed by these observers, some believing that a pattern of maturation in both normal and leukaemic cultures was discernible, others that normal cells matured to a limited extent while acute leukaemic cells did not. Most workers with this fluid medium technique are, however, agreed that its value in the study of normal and abnormal leucopoiesis is greatly restricted by technical difficulties. Total cell counts are made questionable by the difficulty of identifying viable from non-viable cells in the haemocytometer and by the almost invariable presence of cell clumps, and in some cases the demonstrable adherence of nucleated cells, particularly monocytes, to the glass surface of the culture vials makes the whole sampling procedure of doubtful accuracy. Furthermore, differential counts on samples from the cultures are most unreliable, since a very high proportion of cells is disrupted during the preparation of smears and the remaining cells, at least after the first few days, are erratically distributed and many of them are morphologically so distorted as to be almost unrecognizable.

For short periods of study, however, the simple fluid medium technique does provide suspensions of viable cells useful for testing the effects of physical or chemical agents, and for short-term measurements of certain metabolic activities.

Osgood and Bracher (1939) irradiated suspensions of nucleated cells from normal human bone marrow with X-ray doses of 50–2000 r, and observed the changes in cell numbers during the next 7 days. Lymphocytes and early myelocytes decreased in numbers from 24 hours onwards even with doses as small as 50 r. With larger doses the cell decrease was greater, roughly in proportion to the square root of the dose. Morphological changes in irradiated cells were not conspicuous. Transfer of medium from irradiated to non-irradiated cultures did not cause a fall in the number of lymphocytes and immature granulocytes in the non-irradiated cultures, nor did replacement of the medium around irradiated cells alter their pattern of behaviour (Osgood, 1942). An indirect effect of X-rays, at least *in vitro*, was therefore excluded. Gunz (1949*b*) irradiated leukaemic cells *in vitro* and found them susceptible to doses of X-rays within the therapeutic range. He noted a temporary inhibition of mitosis with a decline in the number of immature forms, varying in extent according to the size of the dose given. There was no evidence that the X-rays damaged resting cells, the whole of the observed effect being explicable by action on mitotic or pre-mitotic cells. Abnormal mitoses were produced if irradiation took place some hours after the cultures were set up, but not otherwise.

The effects of chemotherapeutic agents have been studied in fluid culture systems. Salis (1948) found that aminopterin in a concentration of 55 γ per ml. produced little effect on myeloid cells, but Gunz (1949*b*), from a study of mitotic frequency, found aminopterin to be a very powerful mitotic inhibitor, even in extremely low concentrations, producing a complete absence of mitotic figures in the cultures, rather than arrest at some stage of mitosis. The effect was not reversed by folic acid. Osgood and Chu (1948) added urethane in concentrations of 1 in 40,000 to 1 in 200 to cultures of normal and leukaemic human bone-marrow cells. An early but transient increase in mitotic activity occurred, followed by the appearance of nuclear abnormalities, with altered chromatin patterns, nuclear fragmentation and some double nuclei. Gunz (1949*b*) found comparable effects only at concentrations around 1 per cent, and thought this action of urethane *in vitro* to be a direct one on resting cells, probably not providing a parallel with the clinical action of the drug. Both Gunz (1949*b*) and Osgood and Chu (1950) studied the effect of

from normal or chronic leukaemic marrow including the primitive cells present in acute phases of chronic leukaemias. Nevertheless, these workers did not succeed in establishing maintained cultures of any cell strain from normal or acute or chronic leukaemic haemopoietic tissue.

Among other methods used in the attempt to grow human leucocytes on solid media were those of Pierce (1942), who inoculated the chorioallantoic membrane of developing chicks with leukaemic cells, but obtained no definite evidence that the explanted cells were surviving, and of Plum (1952), who implanted normal and leukaemic human marrow in the anterior chamber of the eye of rats, and, after varying intervals, found only mature polymorphonuclear cells present. Although Plum interpreted his results as evidence of normal maturation of leucocyte precursors, whether leukaemic or not, his findings could equally well be due to a mild inflammatory reaction following death of the implanted cells. Equally unsuccessful in giving long-term survival, but enabling direct phase-contrast microscopic studies to be made, was the agar-surface technique used by Pulvertaft and his associates (Pulvertaft, 1952; Pulvertaft and Jayne, 1953; Humble, Jayne and Pulvertaft, 1956). In this method, marrow fragments or pieces of tumour tissue were placed on a plane surface of 3 per cent serum agar on a glass slide, and a coverslip supported on four pillars of soft wax was gently lowered on top of the cells and pressed down until the agar was just distorted. The edges of the preparation were sealed with wax to prevent drying. Direct microscopic examination of various malignant cells and marrow flecks revealed active amoeboid movements and mitotic activity for periods up to 14 days, but haemopoietic cells tended to degenerate without much evidence of proliferation or maturation (Thomas, 1956).

In all the methods hitherto described, solid nutritive media have been provided in or upon which cells might grow and spread with physical support. The possibility that satisfactory growth might occur on simple glass surfaces or in a fluid medium has not, however, been neglected. Culture in fluid media would have the advantage that total and differential cell counts of samples, representative of the whole, could be obtained, and a reasonable quantitative assessment made of changes in numbers of cells present during the period of culture. Moreover, the cytological features could be studied in films prepared and stained by conventional haematological methods.

Osgood and Brownlee (1937) first described a simple method for the propagation of bone-marrow cells in a fluid medium in vaccine bottles. Marrow was aspirated into a citrated salt solution, and the nucleated cells were separated by centrifugation and resuspended in a medium composed of 35 per cent human umbilical-cord serum and 65 per cent Gey's salt solution. The cell suspension was adjusted to a final concentration of 1,000–2,000 nucleated cells per cu. mm. and incubated in small, rubber-capped, vaccine vials. Samples for counting and preparation of stained smears were withdrawn at intervals with a needle and syringe, and the medium could be changed in a similar way after the cells had been allowed to settle. The method, with various minor modifications, was later used by many investigators (Israëls, 1940*a* and *b*; Gunz, 1948*a* and *b*, 1949*a* and *b*; Hoogstraten, 1949; Lajtha, 1950, 1952; Smith, 1952; Blackburn and Lajtha, 1954; Thomas, 1956). In no case was continuous cultivation achieved and the rate of cell degeneration clearly exceeded any proliferation or maturation taking place. Fibroblasts did not appear, and macrophages were only occasionally reported. Various deductions were drawn from

in question forming the bottom of the culture chamber. Smaller vials have also been used, with a microscope slide sealed to the open neck of the vial, the whole being incubated in an inverted position. Cell growth occurs on that portion of the slide forming the bottom of the culture chamber, and removal of the slide enables phase-contrast or stained preparations to be made.

Osgood and Brooke (1955) reported the successful establishment of continuous cultures from peripheral-blood cells of three leukaemic patients. After 296 days *in vitro*, cells derived from a patient with subacute monocytic leukaemia were predominantly like reticulum cells, although many fibroblast-like cells had earlier been present. Occasional giant cells were also present, and some lymphocytic and erythroblastic forms were seen. After 60 days, a culture of cells from chronic myeloid leukaemia showed some rather distorted granulocyte precursors, but also many fibroblast-like "monocytes". All these cells are clearly very considerably different from haemic cells normally seen in blood or bone marrow, but resemble the "macrophages" and "fibroblasts" long known to develop in cultures on solid media.

Berman and his associates (Berman, Stulberg, and Ruddle, 1955; Stulberg, Berman and Ruddle, 1956) have also isolated strains of cells from human marrow, capable of continued growth and readily subcultured. Their method did not involve the calculation of a gradient factor, but was otherwise rather similar to that of Osgood. Cells were grown on the glass surface of 3-ounce medicine bottles, placed on their flat sides, in a fluid medium containing balanced salt solution, human cord serum and embryonic tissue extract. The cultures were observed to pass through three phases, similar to those seen earlier in plasma clot cultures. In the first phase myeloid cells were still identifiable and showed some mitotic activity, but their numbers declined steadily. Large, round, monocytoid or histiocytic cells predominated in the second phase, many becoming attached to the glass. Finally, palisades and networks of spindle-shaped cells resembling fibroblasts and capable of indefinite propagation spread widely over the whole culture field. Among these cells there later appeared, on a few occasions, certain persistent strains of epithelial polygonal cells, arranged in mosaics, not unlike the Hela cells derived by Gey and his co-workers from a carcinoma of the cervix (Gey, Coffman and Kubicek, 1952).

McCulloch and Parker (1956) emphasized the striking morphological alteration invariably taking place in haemic cells cultured *in vitro* before vigorous proliferation and continuous cultivation could be achieved. In their view, both the "Oregon" strains isolated by Osgood and the "Detroit" strains isolated by Berman consist of these "altered" cells. In their own experiments continuous cultivation proved possible with cells derived from leukaemic leucocytes, normal and leukaemic bone marrow, and the bone marrow of rats and mice. Cultures were started by adding whole blood or marrow directly to Carrel flasks containing a chemically defined medium, with heparin as an anticoagulant. After 24 hours' incubation the leucocytes adhered to the glass and the red cells could be aspirated. The medium was then supplemented with 20 per cent horse or human inactivated serum. The specific characteristics of leucocytes were lost within 24 hours, the cells left sticking to the glass being devoid of specific granules and having a large nucleus and scanty cytoplasm. During the next 2 to 8 weeks these cells showed little change apart from spreading over the glass surface, developing more cytoplasm and exhibiting some pleomorphism. The nucleus generally remained central, with one nucleolus, but mitoses were not infrequent

nitrogen mustards on leukaemic cells *in vitro*. Mitotic inhibition and the appearance of abnormal mitoses were observed when concentrations within the therapeutic range were used.

Short-term fluid cultures have been used for metabolic studies by Osgood and his associates (1951), who followed the incorporation of P^{32} into DNA of human leukaemic leucocytes. A small but significant uptake was observed in cultures of acute lymphatic leukaemic cells maintained in culture for 11 days. Lajtha, Oliver and Ellis (1954) similarly studied the uptake of P^{32} and C^{14} in marrow cultures. The blast cells of acute leukaemia were found to have a growth rate much slower than that of promyelocytes and myelocytes of normal marrow, but the rate of DNA synthesis appeared to be the same in cells of the same degree of maturity whether from leukaemic or normal marrow.

Differences in mitotic activity and rate of growth in fluid media containing leukaemic instead of normal serum have not been convincingly demonstrated. Smith (1952) found increased mitotic activity in a marrow culture from one case of acute leukaemia when the cells were incubated in leukaemic serum, but Hoogstraten (1949) and Blackburn and Lajtha (1954) found no difference in the effect of leukaemic as compared with normal sera in fluid medium cultures.

In more recent years, claims to have achieved continuous, long-term cultivation of human blood and bone-marrow cells have been made. Osgood and his associates (Osgood and Krippaehne, 1955; Osgood, 1955; Osgood and Brooke, 1955) introduced a fluid medium technique involving a "gradient" principle, by which the cell concentration and the depth of the cells below the surface are regulated to achieve an optimum for each cell type. The necessary adjustment is determined in the following manner. Heparinized blood or marrow is centrifuged at 4°C. until most of the red cells are deposited, the supernatant plasma containing the nucleated cells is aspirated into another tube, and the cells are centrifuged free from plasma, washed, and resuspended in culture medium made up of 20 per cent human pleural fluid, 30 per cent Difco TC199 and 50 per cent of an acetate balanced salt solution. About 170 ml. of a cell suspension containing between 1,000 and 4,000 cells per cu. mm. are placed in a wide-mouthed, screw-capped, "French square" bottle of one pint capacity, containing a sterile standard microscope slide, slanting across the bottle at an angle of 45 degrees to the horizontal, the upper extremity being just covered by the medium. Four to five per cent CO_2 is added to the air above the culture to give a pH of 7·4. The culture is then mixed thoroughly and left undisturbed in the incubator. Cells are gradually deposited on the slide, in increasing numbers from top to bottom, so that, after a short interval, there will be found small numbers of cells, close to the surface of the medium, at the upper end of the slide, and larger numbers, further away from the surface, at the lower end, with a steady progression in both cell density and depth from the surface between the extremes. At intervals of several days the culture is sampled by removing the slide for staining, mixing the contents of the culture bottle and aspirating 1–2 ml. of the resulting cell suspension for counts. A new sterile slide is inserted at this time. The "gradient factor" is calculated by multiplying the cell count of the mixed culture in thousands per cu. mm. by the depth in cm. of the medium overlying the region of best growth on the slide. This gradient factor is then used to determine the depth of medium appropriate for optimal cell growth to occur over the whole of one large side of a rubber-capped rectangular hard-glass bottle used as a long-term culture flask, the side

After growth in sponge-matrix all six strains proved indistinguishable from one another in histological sections.

In vitro culture methods have therefore failed to establish whether the leukaemic cell is inherently malignant, except in the case of certain animal "leukaemias". They have also been unsuccessful in producing maintained growth and proliferation of leukaemic or normal cells from human blood or marrow, since the strains capable of continuous growth bear only a remote relationship to their parent cells. Perhaps the most important result of all these studies has been the absence of any sharp or consistent difference between the behaviour of leukaemic and normal leucocytes in culture, whether in their initial capacity for differentiation, their early mitotic activity, their tendency to die out, their overgrowth by histiocytic or fibroblast-like elements in solid media or on glass surfaces, or in their eventual transformation into established "altered" cells.

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and occasional giant cells with many nuclei were formed. Despite the occurrence of mitoses, cell numbers gradually declined, and in some cases the cultures died out during this period. In other cultures, however, a sharp alteration in appearance and behaviour of the cells took place after 2 to 8 weeks' incubation. The new cells had a dense and plentiful cytoplasm, and a nucleus with irregular clumps of nucleoplasm. They were pleomorphic, rounding up before mitosis and spreading out when in a resting phase. They multiplied rapidly and soon covered the bottom of the Carrel flask, and could be scraped from the glass, washed, subcultured and maintained *in vitro* indefinitely. Morphologically similar strains of altered cells were isolated from the blood of eight patients with acute myeloid leukaemia, one with subacute myeloid leukaemia and one with chronic myeloid leukaemia, but the cells of chronic myeloid leukaemia usually died out rapidly. Cultures of acute and chronic lymphocytic leukaemic cells were unsuccessful.

Bone-marrow cultures differed from those of peripheral blood in that the prominent early cell was large and spindle-shaped and able to survive and multiply for very long periods, while the dramatic appearance of altered cells tended to occur much later, after 3 to 6 months' continuous cultivation. Cultures of bone marrow were almost invariably successful and cell strains were produced from leukaemias, multiple myeloma and normal marrows. No differences could be seen between the established strains of altered cells derived from cultures of normal or leukaemic marrow.

The nature and relationships of these cells remain uncertain. Repeated attempts to produce malignant changes in animals by injection of altered cells have not been successful, despite the remarkable capacity for proliferation shown by the cells *in vitro*. Although different strains of altered cells are often morphologically similar, there is evidence that they retain an antigenic and functional relationship to their ancestor cells. McCulloch and Parker found that species-specific antibodies were produced in rabbits by repeated injection of altered cells derived from human leucocytes. They also showed that some degree of protection from the lethal effects of irradiation could be brought about by intravenous injection of altered cells into experimental mice. Further information was gained from experiments similar to those of Nowell and his associates (1956), utilizing the fact that rat leucocytes contain alkaline phosphatase, whereas those of the mouse do not. When irradiated mice were treated with intravenously injected altered cells derived from rat marrow, alkaline phosphatase positivity was detectable in the cells of spleen and marrow after 9 to 19 days, although the altered rat cells were themselves phosphatase-negative. The appearance of phosphatase positivity was much slower and the reactions less intense than after the injection of fresh rat-marrow cells, but the altered cells clearly retained a latent capacity to manufacture the enzyme and presumably to participate in recolonization of the irradiated marrow.

The transformation of cells from blood or marrow when grown *in vitro* is not a phenomenon peculiar to haemic tissue, and similarly altered cells derived from other tissues, normal or malignant, may bear a close resemblance to one another and to altered myeloid cells. This feature has become increasingly apparent as more isolated cell strains have been developed. Leighton and his associates (1957), for example, compared three established malignant cell strains from human tumour tissue, one being Osgood's J-96 Oregon strain of monocytic leukaemia, with two "normal" human cell strains and one cell line arising as a morphologically malignant transformation from normal connective tissue.

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energy and of the physical and chemical consequences of ionization has not yet been obtained, although a considerable weight of experimental evidence bearing on the problem has accumulated. Detailed discussion of this work may be found in many review articles and monographs, such as those of Allsopp (1944), Lea (1946), Gray (1946), Bacq and Alexander (1955), and Ellinger (1957). Among the chemical changes of obvious biological importance that have been widely studied *in vitro* are those occurring in water and aqueous solutions, amino acids, proteins, nucleic acids, and enzymes.

The effects of X-rays on water were studied by Duane and Scheuer (1913), who concluded that decomposition into hydrogen and oxygen took place, part of the oxygen remaining in combination with water as hydrogen peroxide. A series of investigations by Risse and by Fricke and his associates showed that X-rays failed to induce the formation of hydrogen peroxide or hydrogen gas in pure water free from dissolved air or other impurities, although the water became chemically activated, probably by splitting into H and OH radicals (Risse, 1929*a* and *b*; Fricke and Brownscombe, 1933; Fricke, 1935; Fricke and Hart, 1936). Dainton (1948) has confirmed the existence of OH radicals in irradiated pure water, but the formation of H atoms still remains suppositious, and the number of free radicals produced by a given dose of X-ray has not been determined. In the aerated solutions of chief biological interest there seems little doubt that OH and HO_2 radicals are formed and that reduction of the latter with formation of hydrogen peroxide readily takes place. The oxidizing and dehydrogenating capacity of these radicals may explain certain of the changes which many organic substances have been found to undergo when irradiated in aerated aqueous solutions, since these changes usually involve oxidation and release of hydrogen.

Amino acids dissolved in water are usually deaminated by X-irradiation whether oxygen is present or not, the alpha-amino group being probably replaced by an OH radical; amino groups in other positions are not commonly affected. Changes in cysteine and glutathione are of particular interest in view of the special importance of these amino acids in leucocyte metabolism (see Chapter 6). Kinsey (1935) initially described the radiochemical destruction of glutathione. Barron and his associates (1949) claimed that oxidation of —SH groups to disulphide was the major radiation effect, but Dale and Davies (1951) showed that hydrogen sulphide was also formed, the yield varying with pH. Deamination of these sulphydryl compounds does not occur, although the related amino acid cystine is deaminated.

Little precise chemical information is yet available about changes in the amino acid composition of proteins after ionization, but certainly extensive chemical and physicochemical alterations take place within the protein molecule. Nearly all proteins are denatured and can be precipitated at the isoelectric point, while changes in viscosity, refractive index and other physical properties occur. Most experimental evidence is consistent with the hypothesis that the free radicals of activated water bring about the transformation of the protein molecule by splitting the peptide chain or causing unfolding and an increased tendency to aggregation, with the production of some smaller molecules and some of increased molecular weight.

Irradiation of DNA solutions leads to a sharp decrease in their characteristically high viscosity (Sparrow and Rosenfeld, 1946), a change due to depolymerization by the breaking of main-chain bonds (Taylor, Greenstein and Hollaender, 1948). Free radicals from the

CHAPTER 9

GENERAL PRINCIPLES OF THERAPY IN LEUKAEMIA

I. Radiation by X-rays and Radioactive Isotopes

THE efficacy of X-rays in the treatment of certain forms of leukaemia and allied diseases was demonstrated early in this century (Pusey, 1902; Senn, 1903), and for nearly fifty years radiation therapy provided the chief means of control in chronic leukaemias. Acute leukaemias failed to respond and were sometimes exacerbated by X-ray treatment. Remissions in chronic leukaemias were often very complete but relapse invariably occurred, and, indeed, statistical surveys on large numbers of treated and untreated patients showed that therapy did not greatly prolong life, although incapacity was much reduced during the years of survival (Minot, Buckman and Isaacs, 1924; Hoffman and Craver, 1931).

Satisfactory clinical and haematological remissions in chronic leukaemias have also been brought about by internal irradiation with radioactive phosphorus (Lawrence, Scott and Tuttle, 1939), but reduction of splenomegaly and lymphadenopathy is less well achieved than by external irradiation (Reinhard *et al.*, 1946). An advantage of radioactive isotope treatment is the freedom from symptoms of radiation sickness, but, nevertheless, external irradiation has generally been preferred.

The striking ability of irradiation, whether external or internal, to induce remissions in chronic leukaemias but not in acute ones, the development of general remissions after irradiation of quite small areas, the eventual production of a resistant state, and many other phenomena observed during the clinical management of leukaemic patients have stimulated a wealth of experimental work on the mode of action of radiations in leukaemia. It is appropriate here to review these studies briefly against the general background of knowledge of the biological effects of radiation, and to discuss recent attempts to combine massive radiation destruction of haemic tissues with protective transplants of haemopoietic cells.

Biological effects of ionizing radiation

The general effects of exposure of cells and tissues to electromagnetic radiations such as conventional X-rays or gamma-rays, or to corpuscular emanations such as alpha- or beta-particles, are qualitatively very similar, and depend upon changes resulting from the absorption of radiant energy within the cells, with the production of electrically charged atoms and molecules. Different types of radiation vary in their ability to penetrate tissues and in the extent to which they stimulate the formation of charged particles or ions, properties which appear to be reciprocally related, the most penetrating radiations having the lowest ionizing power, but the biological consequences of ionization, however induced, are much the same. A full understanding of the molecular changes caused by radiant

cells contribute to the development of nuclear degenerations (Duryee, 1939, 1949). Cell death may result from interference with vital metabolic processes leading to premature senescence, or may follow upon nuclear damage or the induction of lethal mutations. Very high doses of radiation produce immediate coagulation necrosis.

When tissues in the intact animal are irradiated the direct effects on cells within the beam are supplemented by indirect radiation effects in neighbouring tissues whose blood supply has been injured by direct action. The cell degeneration resulting from damage to the blood supply is most prominent in actively proliferating tissues, but affects also resting and differentiated tissues, and in this respect the indirect action of radiation is less selectively exerted upon dividing cells than is the direct action. A further manifestation of radiation damage in the intact animal is a constitutional effect, radiation shock, resulting from the widespread circulation of toxic substances produced by the breakdown of cells directly exposed. This effect is indiscriminate in distribution.

Very wide differences exist between the relative cytological and functional responses made by various cells and tissues to a given dose of radiation. Bergonié and Tribondeau (1906) formulated a general law of fundamental importance, that "les rayons X agissent avec d'autant plus d'intensité sur les cellules, que l'activité réproductrice des cellules est plus grande, que leurs devenir karyokinetique est plus long, que leur morphologie et leurs fonctions sont moins definément fixées". This law generally holds true for all types of radiation; the sensitivity of cells is proportional to their mitotic activity and inversely proportional to their degree of differentiation, at least in so far as related cells having a common derivation are concerned. Different tissues of distinct origin and more remote relationship show varying degrees of radio-sensitivity, less clearly in accord with their relative reproductive activities and state of differentiation. Extensive observation of radiation effects in man suggests that different cell types may be arranged roughly in the following order of decreasing sensitivity: lymphoid cells, other haemopoietic cells, including erythroblasts, myeloblasts, granulocytes and megakaryocytes, epithelial cells, endothelium, connective tissue, bone cells, nerve and brain cells, muscle cells. The blood-forming organs are thus the most radio-sensitive of tissues, and the changes occurring in them after irradiation have been the subject of numerous investigations both in experimental animals and in man.

Effects and mode of action of X-rays on normal and leukaemic haemopoietic tissues

In a series of detailed reports, Heineke (1903, 1904*a* and *b*) described the destructive effects of radiation on cells of the thymus, spleen, lymph glands and bone marrow in the intact animal. Nuclear fragmentation and pycnosis were apparent within a few hours after exposure and reached a maximum at about 12 hours, the cells involved being lymphoblasts and lymphocytes and, less conspicuously, all other precursor cells. Thymus, spleen and lymph glands shrunk greatly as a result of the destruction of lymphatic tissue, and a progressive depopulation of the bone marrow was observed, terminating in complete aplasia after 5 or 6 days. When sublethal doses were used, a phase of hypocellularity was followed by regenerative activity after the sixth day with return to normal in 2 to 3 weeks. These fundamental studies were subsequently confirmed by many workers. Among a

solvent water are probably again responsible for this reaction. It is likely that comparable depolymerization of RNA may also be brought about by radiation (Grinnan and Mosher, 1951), and that radiosensitivity of nucleic acids *in vivo* is at least as great as *in vitro* (Limperos and Mosher, 1950).

Most enzymes are inactivated by ionizing radiations, an effect which takes place in a consistent manner when purified enzymes are used in aqueous solution. The studies of Dale (1940, 1942, 1943) showed that, in these circumstances, the extent of inactivation was proportional to the amount of radiant energy absorbed by the whole solution. Thus a given dose of X-rays inactivated the same total amount of enzyme whether in high or low concentration in the solvent water, a state of affairs most readily explicable as a consequence of the action of free radicals derived from activated solvent. Barron and his associates (1949) regarded oxidation of sulphydryl groups by OH and HO_2 radicals as the mechanism of inactivation of enzymes containing —SH groups, such as phosphoglyceraldehyde dehydrogenase, adenosinetriphosphatase and succinoxidase, since these enzymes could be reactivated by the addition of glutathione. Glutathione in high concentration was also shown to protect ribonuclease from inactivation by radiation (Holmes, 1950). The doses of radiation used in many of these experiments on enzyme inactivation *in vitro* were within the therapeutic range, but the extent to which similar inactivation takes place *in vivo* is undetermined. Simple comparisons are clearly impossible since the test-tube conditions, with pure enzymes in simple aqueous solution, differ so greatly from those in living cells, with the enzymes in a remarkably complex metabolic environment. Indeed, few changes in general enzymatic and metabolic activity have been observed to occur consistently immediately after irradiation *in vivo*. After minimal lethal doses of X-rays an increase in both anabolism and catabolism has been found in haemopoietic tissues (Richmond, Altman and Salomon, 1951; Altman *et al.*, 1951). Klein and Forssberg (1954) and other investigators have reported that DNA synthesis is inhibited by moderate doses of radiation, whereas the synthesis of RNA and of other proteins is unaffected. There is evidence that the inhibitory effect on DNA formation may not be due to direct interference with any particular step in the synthetic process, but may result from disturbance of some cellular mechanism during the resting presynthetic phase (Lajtha, 1957). Absorption of carbohydrates, fats and proteins continues, and cell respiration and glycolysis are normal. Biochemical lesions produced by radiation *in vitro* have therefore not yet been correlated with those caused *in vivo*.

When living cells are irradiated the most obvious morphological and functional effects are observed in the nucleus and in the process of cell division. Low doses appear to inhibit mitosis for a period, while increasing doses produce a more complete mitotic inhibition followed by chromosomal degenerations of several kinds. Perhaps as a result of depolymerization of nucleic acids the chromosomes exhibit increased stickiness and a tendency to clumping and pycnosis. Breaks in chromosomes or in separated chromatids also occur, and subsequent recombination of fragments may involve translocations, deletions, and other genetically important phenomena. Gross nuclear pycnosis or karyorrhexis may be found in resting cells, but cell sensitivity is much enhanced during the mitotic phases. The mechanism of chromosome breakage is still obscure, but a biochemical explanation is more likely than a mechanical one. Isolated cell nuclei are relatively radio-resistant, and it seems probable that toxic substances produced in the cytoplasm of irradiated intact

evidence of the importance of indirect effects. Good remissions in chronic myeloid leukaemia can be brought about by irradiation of the spleen alone, and both the peripheral leucocyte count and the bone-marrow differential cytology return towards normal (Parsons *et al.*, 1954). The extent and duration of remissions in chronic leukaemia following X-ray treatment seem to be much the same whether treatment is given to the spleen alone, or to the spleen and long bones, or to the whole body (Hoffman and Craver, 1931; Medinger and Craver, 1942). The disappearance of distant leukaemic infiltrations, retinitis and priapism, and reduction in lymph-node enlargement have frequently been observed to follow local splenic irradiation and these effects must clearly be due to an indirect mechanism. Direct damage to circulating leucocytes or to leucopoietic tissue may certainly occur as a result of local exposure of the spleen or other regions of the body. During the period of irradiation of any organ a substantial part of the circulating leucocyte mass is likely to pass through it and may undergo changes of greater or less degree. Blotner and Sosman (1944) found a drop in the leucocyte count after irradiation of the praecordium over the left ventricle, and Abels *et al.* (1941) reported a rise in the organic acid-soluble phosphate content of leucocytes in leukaemia after similar X-ray treatment to the praecordium. It is difficult in experiments such as these to exclude exposure of some haemopoietic tissue in sternum, ribs, or vertebrae, but it appears likely that a chemical change in leucocytes, perhaps due to interference with nucleoprotein metabolism, is caused directly by irradiation of the large volume of blood passing through the heart during the time of exposure. Nevertheless, direct action of this kind does not provide an acceptable explanation for the regression of distant leukaemic deposits and the general change in leucopoietic activity after irradiation of relatively small areas.

Attempts to demonstrate the existence of toxic substances, inimical to leukaemic proliferation, circulating widely throughout the body after local X-ray treatment have not been convincingly successful. When healthy animals were injected with serum from irradiated animals or leukaemic patients no consistent leucocyte changes were found (Klieneberger and Zoeppritz, 1906), although some observers reported transient leucopenia (Grawitz, 1904; Capps and Smith, 1907). The effects of irradiating one of a pair of parabiotic animals were studied by Zacherol (1926) and by Barnes and Furth (1943). Severe radiation effects, with terminal aplasia, were found in the exposed partner, and similar though less severe changes occurred in the unirradiated partner after a short delay. The authors attributed these changes to humoral factors. Lawrence, Valentine and Dowdy (1948) set up carotid artery anastomoses between pairs of cats shortly before, or at varying intervals up to 82 hours after, irradiation of one partner. Cross-circulation was maintained for periods of 2 to 10 hours. Detailed haematological studies on the unexposed animals were carried out for 28 days after the start of the experiment, and in no case was there a significant fall in the leucocyte count or other evidence of deleterious indirect radiation effect, despite the fact that the dosage employed resulted in the death of the exposed cats within 4 to 7 days. The operative procedure of establishing cross-circulation induced the expected leucocytosis for a few days and this was observed to be more marked in some animals whose partners were irradiated immediately before or after the operation. This was the only change noted which might have been secondary to the circulation of metabolites derived from tissue breakdown. The authors concluded that the existence of a specific indirect radiation effect on the haemopoietic system, mediated by circulating

number of excellent review articles and monographs covering this field of research are those of Selling and Osgood (1938), Warren and Dunlap (1942), Denstad (1943), Bloom (1948), and Ellinger (1957). Some confusion arose in interpreting the earlier studies because various animal species, differing at least quantitatively in their response to given exposures, were used, and because cytological comparisons between biopsy and post-mortem materials were not easy, particularly since the classification and recognition of precursor cells of the haemopoietic system were often in dispute. The failure of many early workers to recognize primitive basophilic erythroblasts, for example, led them to consider erythropoietic cells as radio-resistant, since haemoglobinized erythroblasts were not readily destroyed. When primitive erythroblasts were distinguished from early lymphocytic and myeloid cells, however, they were found to be at least as sensitive as granulocyte precursors or megakaryocytes. A further problem concerned the production of radiation effects in haemopoietic organs by total-body exposure as compared with local irradiation. The general range of cell destruction brought about by the two methods was qualitatively the same, but with equal doses much greater effects followed total-body irradiation, indicating the importance of indirect action in the overall response of the intact animal. The indirect effect was also manifest in hypoplastic changes occurring in haemopoietic tissues not themselves directly exposed (Le Blond and Segal, 1942).

In view of the difficulties in separating direct and indirect effects *in vivo*, and in view also of the small number of observations possible in healthy human beings, attempts have been made to assess the radio-sensitivity of normal human blood and bone-marrow cells *in vitro*. The studies of Osgood and Bracher (1939), of Osgood (1942), and of Gunz (1949), described in Chapter 8, showed that X-ray doses within the therapeutic range acted *in vitro* principally by inhibiting mitosis in haemopoietic cells. Indirect action by substances released in the medium could not be clearly demonstrated, and mature and resting blood cells were radio-resistant. The rapid fall in the peripheral leucocyte count found so often after local or whole-body exposure to therapeutic doses has, of course, long been thought to be secondary to some action other than direct leucocyte destruction (Lacassagne and Lavedan, 1924; Jolly, 1925). Possible mechanisms include damage to leucopoietic cells (Aubertin and Beaujard, 1905), the action of toxic materials formed elsewhere (Helber and Linser, 1905; Zwerg, 1932) and the emigration of white cells from the blood (Trowell, 1952). These secondary mechanisms must all be borne in mind when the effects of radiation in leukaemia are considered.

From the studies of radiation effects on normal animals and tissues, discussed above, it might be expected that ionizing radiations would influence leukaemic processes by a direct action on the actively proliferating leukaemic cells in the sites exposed and by an indirect action on more distant cells due to alteration in their chemical environment resulting from release of tissue breakdown products into the circulation. The sensitivity of leukaemic cells *in vitro* to direct radiation in therapeutic doses has been established and it has also been shown that cells derived from an irradiated leukaemic patient may have diminished proliferative ability in culture (Gunz, 1949). Leukaemic cells appear to be more radio-sensitive than normal cells of comparable maturity (Osgood, 1940), while among the leukaemic leucocytes the least mature appear most sensitive, in agreement with Bergonié and Tribondeau's law (Piney and Riach, 1932).

The clinical and haematological response of irradiated leukaemic patients provides

Haemopoietic recovery after irradiation in leukaemia

The emergence of haemotological remission after radiation has checked the leukaemic overgrowth depends upon the ability of residual normal haemopoietic cells to resume full activity, repopulate the marrow, and produce normal cells for release into the circulating blood. This is a paramount requirement, and the failure to achieve remissions in acute leukaemia after radiation-induced destruction of the highly sensitive leucoblastic tissue appears to be due to an inability of such normal cell precursors as remain to resume their haemopoietic role, so that an aplastic, thrombocytopenic state ensues and the patient is clinically no better and perhaps worse.

The eventual failure of radiation in chronic leukaemias that responded initially remains unexplained. As in the case of resistance to chemotherapy, some alteration in metabolic processes within the leukaemic cells may occur, rendering them less susceptible to the chemical basis of radiotherapeutic action. The increased doses then necessary to achieve an effect on the leukaemic cells are too large to spare normal haemic cells, and continued radiation therapy becomes impracticable. This problem is of course fundamental in the successful use of radiation in whatever therapeutic sphere; damage to malignant cells must be brought about without gross concurrent damage to normal tissues, a requirement more difficult to satisfy in diseases of the blood-forming organs than in most other regions because of the high radio-sensitivity of normal haemopoietic cells. Recent studies of protection against radiation damage and of methods enabling recovery from otherwise fatal irradiation to take place are therefore of the highest potential importance in the field of leukaemia. These studies, reviewed in Chapter 4, established that recovery took place as a result of recolonization of haemopoietic sites, whose cellular contents had been destroyed by radiation, by haemic cells from a screened organ or from a donor animal. Attempts to apply this work to the practical therapy of leukaemia in animals and man are being actively pursued at present and a few preliminary results have already been reported.

Hollcroft, Lorenz, Congdon and Jackson (1953) first used lethal doses of X-rays followed by bone-marrow injections in the treatment of experimental leukaemia in guinea-pigs. The method was unsuccessful, although these workers did obtain some apparent cures of localized lymphosarcoma in mice by a similar technique. Barnes *et al.* (1956) worked with a generalized lymphoid leukaemia of the CBA mouse which could be transmitted by intravenous or subcutaneous cell passage. One week after inoculation with leukaemic cells mice were given an X-ray dose of 950 rad, being the LD98 for normal CBA mice, and then treated with intravenous injections of isologous myeloid tissue (from the same inbred strain) to allow recolonization of haemopoietic and lymphopoietic tissue to take place. The animals recovered from the acute effects of radiation, but the leukaemic cells had not been entirely eradicated, and death from generalized leukaemia occurred after about a month. When, however, comparable mice were irradiated over 25 hours with the larger dose of 1,500 rad, and then given isologous myeloid tissue, 25 out of 35 animals were still surviving after 3 months. In further, more extensive experiments, Barnes and Loutit (1957) explored a second possible approach to the successful treatment of murine leukaemia. Their earlier studies had presupposed that leukaemic cells were more radio-sensitive than normal lymphocytes or bone-marrow cells and might be completely destroyed by an LD98 X-ray dose, recovery then being brought about by injection of isologous bone marrow or infant spleen. This had proved not to be the case, and excessive

toxins, was disproved by their experimental results, although admitting that more prolonged cross-circulation might have permitted additional changes to emerge. Several workers have used *in-vitro* methods in the search for leucotoxic activity in the plasma of irradiated leukaemic patients, by observing the effect of such plasma on normal or leukaemic cell suspensions and comparing it with that of plasma from unirradiated leukaemic patients and from normal subjects (Capps and Smith, 1907; Billings, 1922; Gunz, 1949). Some evidence emerged in favour of leucotoxic activity, but most of the experimental results were difficult to interpret clearly and no firm conclusions could be drawn.

While no proof of the production of leucotoxins by irradiation has been forthcoming, there is experimental support for the mediation of some indirect effects by hormonal or other physiological mechanisms. Selye (1946) emphasized that many of the metabolic changes observed after radiation were similar to those following other injurious stimuli, such as traumatic shock, burns, and exposure to cold or excessive heat. In each case hyperglycaemia, rise in blood ketones, fall in blood cholesterol, increase in blood non-protein nitrogen, and changes in acid-base balance occurred. Selye regarded these changes as attributable to a non-specific stimulation of the pituitary–adrenal cortical system. Increased activity of this system was thought to be responsible also for involution of thymic and lymphoid tissue after radiation, and Dougherty and White (1946) showed that small doses of X-ray producing typical changes in normal animals failed to produce these effects in adrenalectomized animals. The temporary rise in the leucocyte count, involving chiefly granulocytes, often observed shortly after X-ray exposure, can be explained by the same mechanism.

A second physiological mechanism of potential importance in mediating indirect changes following irradiation is the control over maturation and release of haemic cells exerted by the spleen. The functions of the spleen in this regard are complex and there is no certain proof of the existence of a splenic hormone controlling haemopoiesis, but numerous clinical and experimental observations strongly suggest that the spleen helps to maintain the normal balance of production and release of red cells, granulocytes and platelets (Hayhoe and Whitby, 1955). This balance is certainly gravely disturbed in leukaemia and some part of the imbalance may be due to altered function of the hypertrophied spleen. Splenectomy in leukaemia is sometimes followed by changes in the blood resembling those produced by irradiation, although they are usually of short duration and do not greatly modify the leukaemic process (Astaldi and Ravetta, 1948). Alteration in splenic function after X-ray treatment may, therefore, contribute to the general effect, but the contribution seems unlikely to be a major one.

In sum, the chief established mechanism of radiation action in the leukaemias is a direct one upon the proliferating leucocytes, involving interruption of mitosis. This effect leads to decreased production of leukaemic cells and allows normal haemopoiesis to become re-established. Irradiation of the enlarged spleen, or spray irradiation of a large part of the skeleton, exposes a considerable bulk of sensitive leukaemic tissue to mitotic damage, including both the leucopoietic sites themselves and the peripheral blood cells of varying stages of maturity passing through irradiated areas throughout the time of exposure. Specific indirect "leucotoxic" action remains unproven, but non-specific indirect effects due to stimulation of the pituitary–adrenocortical stress mechanism probably contribute to the overall response.

various skeletal sites in the cadaver within 1 or 2 hours of death. Aspiration biopsy of the ilium also provides a possible source of material, although several aspirations would probably be necessary to yield the required number of cells. Marrow cells can be stored for more than 80 days by the Polge technique of freezing to −80°C. in glycerol and remain effective (Barnes and Loutit, 1955). That quantities of stored marrow cells of the necessary order can be given with safety in man, and may establish themselves at least for short periods, has been demonstrated by Thomas *et al.* (1957), Tocantins (1958), and others. Technical details of importance in the acquisition and injection of marrow include the very careful observance of a strictly aseptic procedure, the avoidance of cell clumping by the use of heparin or enzymes, the preservation of cell viability and integrity by gentle and expeditious collection of samples, and the use of 5 per cent albumin as a protective additive. When due attention is paid to these details, marrow collection and transplantation in man becomes a reasonably practicable procedure.

The most difficult problems, however, are immunological ones. In experimental animals recovery from lethal doses of X-ray may be complete and permanent when the donor marrow cells are isologous, but when homologous or heterologous cells are used recovery is often only temporary and after a few weeks severe reactive changes in lymph nodes and spleen lead to extreme atrophy of these organs, steady loss of weight occurs, and the animals die (Congdon and Urso, 1957). This sequence of changes has been interpreted generally in the following manner. Heavy total-body irradiation damages not only the haemopoietic and lymphopoietic tissue, but also the antibody-forming system. The animal is therefore incapable of developing an immune response and for this reason the homograft or heterograft successfully takes. After a period of time varying according to the radiation dose the immune mechanism recovers and tends to reject the foreign transplant. In addition, the transplanted cells may develop an immunological reaction against the tissue cells of the host. Whether the first or second of these immune mechanisms predominates is unsettled (Makinodan, 1958; Trentin, 1958), but clearly an intolerable immunological situation is brought about and proves fatal to the host. Antibody formation by the graft might be minimized if foetal rather than adult cells were injected, since foetal reticulo-endothelial elements may actively acquire tolerance to the host (Billingham, Brent and Medawar, 1953, 1955), and there is evidence that use of such tissues decreases the incidence of foreign-marrow reactions in mice (Congdon and Urso, 1957). Immunity problems in the clinical application of bone-marrow protection against irradiation injury in a species genetically so heterogeneous as man are theoretically much more difficult to solve than are those arising in experiments with laboratory animals. Normal autologous or isologous marrow cells are not available, except in the rare case of identical twins, and homologous material from human donors is of infinitely variable genetic constitution. Moreover, early rejection of the transplant by the host is likely to occur when sub-lethal doses of radiation are used, since the immune mechanism retains some functional capacity, and, of course, fully lethal X-ray doses can hardly be contemplated during the early stages of clinical trial of these techniques in man. Alternative methods of reducing the immune response, less drastic and irrevocable than lethal irradiation, are therefore being sought. Cortisone has long been known to facilitate tumour transplantation, presumably by reducing antibody production in the host (Howes, 1951), and it has been used successfully in combination with irradiation to permit the transfer of human

irradiation had to be used before the disease could be eradicated in a proportion of animals. The possibility was therefore considered that administration of homologous marrow (from a different strain within the same species) might be more effective by producing at the same time the recolonization necessary for recovery from acute radiation sickness and also an immune reaction directed against surviving leukaemic cells having the tissue specificity of the host. An attempt was made to exaggerate the latter action by using homologous tissue from animals previously immunized against CBA leukaemic cells. The results were not conclusive. Some evidence emerged that residual leukaemic cells may have been destroyed by an immune response of the homografts, since the mice treated with homologous material from either normal and immunized animals survived longer than those given isologous tissue and did not show post-mortem signs of leukaemia. Nevertheless, these mice all developed diarrhoea and gross malnutrition and died in a wasted, dehydrated condition, and any residual leukaemic cells may have failed to grow in such poorly nourished hosts. Why functional gut disturbance should be so prominent in animals treated with homologous cells is not clear. Neither immune activity of the homograft against the host, nor a restoration of the host's immune responses directed against the graft would be expected to involve principally the intestinal tract. The authors therefore postulated that intestinal radiation damage was the fundamental defect and that foreign cells were less well able to hold this in check than were isologous cells.

Experiments on similar lines with leukaemias of animals other than rodents have not yet been reported, but protection from radiation death by implantation of homologous bone marrow has been successfully achieved in some higher species, though not in others. Thus while Crouch and Overman (1957) described satisfactory protection by homologous marrow in monkeys, Alpen and Baum (1958) found homologous marrow implants ineffective in protecting irradiated dogs, although autologous implants were successful.

A uniformly successful technique for treating animal leukaemia by radiation and cell inoculation has not yet been devised, and the problems arising when extension of this work to human patients is contemplated are formidable. In the first place a dose of total-body irradiation sufficient to kill the leukaemic cells without permanently damaging vital tissues elsewhere in the body must be used. The sensitivity of the gastro-intestinal tract and other tissues made up of actively proliferating cells certainly introduces a limiting factor in dosage, and it is still questionable whether the necessary destruction of all leukaemic tissue can be achieved without so severely damaging other sensitive cells that recovery is impossible. The protection against radiation effects conferred by some reducing agents such as glutathione and AET (S,2-aminoethylisothiuronium-Br-HBr) applies to all tissues penetrated by the agents. A way of protecting normal tissues with such chemicals while leaving leukaemic cells unprotected has not yet been found, but research in this field may lead to a solution to the problem of total dosage.

A second difficulty, more easily overcome, concerns the provision of adequate quantities of marrow cells for transplantation in man. Evidence from a number of sources (van Bekkum, 1958) suggests that all cell types present in normal marrow are required for optimum response, the more primitive cells being of chief importance, and that intravenous injection of some 30 to 40 billion mixed bone-marrow cells would be the minimum quantity required for repopulation of the haemopoietic system in man. Such an amount of marrow could be obtained from 4 to 5 adult ribs removed at thoracotomy or from

half-life" (the time taken for the original level of radioactivity to be reduced to half) and on the rate of excretion, measured as the "physiological half-life". In addition, certain radioisotopes are not uniformly distributed about the body, but are selectively concentrated in specific sites. This property provides the chief justification for the use of radioisotopes in therapy, and the ideal agent would be one selectively absorbed in malignant cells and having a half-life long enough to ensure their destruction, but not so long as to constitute a subsequent menace to surrounding normal tissues. Unfortunately, no radioisotope conforms to these requirements. The most effective at present in the therapy of leukaemias and polycythaemia is radiophosphorus, P^{32}. This isotope is produced artificially in a cyclotron or nuclear reactor, and has a physical half-life of 14·3 days. It emits beta-particles (electrons) with a maximum range in tissues of less than 1 cm. P^{32} is given by mouth or intravenously as an isotonic solution of sodium phosphate and is distributed widely about the body by the blood stream. Low-Beer, Blais and Scofield (1952) found it most concentrated in bone, with successively smaller concentrations in spleen, liver, kidney, muscle, blood, fat, skin and nerve tissue. Various malignant cells, including those of lymphoma, lymphosarcoma and leukaemia, exhibit a high uptake of the isotope (Jones, Chaikoff and Lawrence, 1940; Osgood and Tivey, 1950). P^{32} is excreted in the faeces after oral administration, between 15 and 50 per cent being lost in this way. Absorbed or intravenously administered P^{32} is gradually excreted through the kidneys, the biological half-life in the blood of leukaemic patients being approximately 8 days (Tivey and Osgood, 1950). In leukaemia and polycythaemia P^{32} acts as a powerful inhibitor of haemopoiesis, and overdosage leads to medullary aplasia with anaemia, agranulocytosis and thrombocytopenia. There is a certain unpredictability of response in individual patients, whose susceptibility to the action of the isotope varies considerably, and this appears to be a more striking feature in leukaemia than in polycythaemia. It has become customary, therefore, when P^{32} is used in the treatment of chronic myeloid leukaemia, to give small doses of 1 to 3 mc weekly for 4 to 6 weeks rather than a single massive dose.

Since beta-rays have so short a penetration, radiation effects are principally found in bone-marrow, spleen and malignant cells in which the isotope is concentrated, and patients do not suffer from radiation sickness during treatment and can be controlled without admission to hospital. Despite the theoretical and practical advantages of P^{32}, it has not proved more satisfactory than X-rays or chemotherapy in the management of chronic leukaemia and its use in most centres is now confined to the treatment of polycythaemia vera. P^{32}, like external irradiation, is unsuitable for use in acute leukaemia.

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cancer cells to rodents (Herbut and Kraemer, 1956*a*). The role of the properdin system in resistance to cell transplants is also being actively explored. Properdin, a serum globulin that attacks bacteria and viruses and lyses red cells in the presence of complement and magnesium ions, was described by Pillemer and associates in 1954, and may perhaps be identical with a guinea-pig serum component earlier shown by Kidd (1953) to inhibit the growth of transplanted lymphoid tumours in mice. Zymosan, an insoluble carbohydrate derived from yeast cells, has been found to combine with properdin *in vitro* and *in vivo*, producing an inactive complex (Pillemer *et al.*, 1955), and Herbut and Kraemer (1956*b*) found that preliminary intravenous injections of zymosan before transplantation of human carcinoma cells into rats led to a proportion of successful takes, presumably by reducing that part of the host resistance due to properdin. Application of this technique in the treatment of leukaemia by irradiation and marrow grafting may help to overcome the initial resistance to transplanted cells in sub-lethally irradiated animals, and allow another step forward to be made towards the eventual clinical objective.

Combination therapy with X-rays and chemicals

While the principles of treatment of leukaemia with chemical agents will be discussed in the next chapter, it is pertinent to refer here to the attempts which have been made to increase the selective efficacy of ionizing radiations by the concurrent administration of chemicals. Bane, Conrad and Tarnowski (1957), in a review of this field, classified these attempts into three groups: combination of radiation with specifically anti-tumour agents in a search for synergism, tumour sensitization with non-carcinostatic agents, and introduction into the neoplasm of chemicals capable of increasing radiation dosage by secondary radiation, such as certain heavy metals. Methods in the first group have not proved generally advantageous in the management of leukaemia, and this was perhaps to be expected since the difficulties in radiation control of leukaemias are due not so much to resistance of leukaemic cells to destruction by appropriate doses, as to the fact that such doses may severely damage normal haemopoietic cells at the same time. Combined use of radiation and cytotoxic drugs tends to exaggerate this effect and readily leads to medullary aplasia (Wilkinson, 1955). Attempts to increase the radio-sensitivity of tumour cells by increasing oxygen tension (Gray *et al.*, 1953), or by injecting synkavit (tetrasodium-2-methyl-1,4-naphthohydroquinone phosphate) intravenously before exposure (Mitchell, 1948, 1953), while offering some promise of improvement in solid tumour response, have not been used extensively in leukaemia at present. The same is largely true of secondary radiators, but if such substances could be found to localize selectively in leukaemic cells and not in normal leucopoietic tissue, and if the complex problems of control of secondary radiation dosage could be solved, their use would certainly be of the greatest potential value.

Mode of action and effects of internal sources of ionizing radiation

Radioactive substances introduced into the body exert the same general biological effects of ionization as do external radiation sources, but they differ from X-rays in several therapeutically important respects. These include the type of radiation emitted and its duration, which depends on the rate of radioactive decay expressed by the "physical

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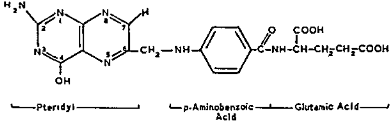
from blood transfusion, since whole blood or plasma transfusions may lead to regression of acute leukaemia in a proportion of cases.

ANTIMETABOLITES

The Folic Acid Antagonists

Extensive nutritional and microbiological studies over a period of nearly twenty years culminated in the identification and synthesis of pteroylglutamic acid, or folic acid, by Angier *et al.* in 1946. This substance, one of the B group of vitamins, is closely concerned in cell metabolism, particularly in the processes of cell growth and division. Deficiency leads to macrocytic anaemia, often with megaloblastic erythropoiesis, to leucopenia, and to certain gastro-intestinal changes manifest by gingivitis, sore tongue and diarrhoea. The complex chemical structure of folic acid is given below (Fig. 17). Although pteroylglutamic acid occurs naturally as such, it is commonly found in conjugated form with up to seven glutamic acid molecules incorporated in the structure, and the action of tissue



PTEROYLGLUTAMIC ACID (FOLIC ACID)

FIG. 17.

conjugases is necessary to release the vitamin from these conjugates. Folic acid itself does not appear to be an active substance in cytopoiesis, but is the precursor of a highly active derivative known as "folinic acid" or the "citrovorum factor" which is produced by enzymatic hydrogenation and the insertion of a formyl group into folic acid.

The conversion of folic to folinic acid is an essential change if the vitamin is to function normally, and although the process of conversion and the mode of action of folinic acid are biochemically complex (see the extensive discussions of Nichol and Welch, 1950; Welch and Heinle, 1951; Welch and Nichol, 1952; Nichol, 1953), they are most relevant chemotherapeutically since the action of folic acid antagonists used in the treatment of leukaemia is primarily to block this conversion and so prevent the participation of folinic acid in cell metabolism (Blakley, 1954). Conversion is an enzymatic process occurring chiefly in the liver and bone marrow and requiring the presence of ascorbic acid as a hydrogen donor. Tetrahydrofolic acid and folinic acid (Figs. 18 and 19) probably act as prosthetic groups in enzymes concerned with the formate pool of one-carbon units. Important reactions in which they are involved include the methylation of pyrimidine to thymine, ethanolamine to choline, and homocysteine to methionine, and most important in the context of nucleic acid biosynthesis, the insertion of the 2- and 8-carbon atoms into the purine ring (Sonne, Buchanan and Delluva, 1948; Greenberg, 1954). These and other

CHAPTER 10

GENERAL PRINCIPLES OF THERAPY

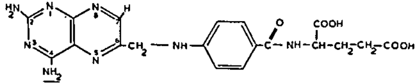
II. Chemotherapy. Development of Chemotherapeutic Agents. Their Modes of Action and Usage

WHILE the practical value, if not the mode of action, of orthodox radiotherapy in the control of leukaemia has become clearly established after the experience of more than half a century, the potentialities of chemotherapeutic agents are far less well assessed. Rapid progress during the last decade in the development and trial of such substances has provided an almost bewildering variety of antimetabolites and cytotoxic drugs, which, together with the adrenocortical hormones, are claiming increasing attention from the physician engaged in treating leukaemic patients. In this chapter the nature, mode of action and general principles of usage of each of the chief groups of available chemotherapeutic drugs used in leukaemia will be reviewed briefly. Consideration of the most appropriate chemotherapeutic or radiotherapeutic approach to each of the major varieties of leukaemia will be found in the later chapters dealing with those diseases in turn.

The cytotoxic drugs appear to be active by entering the cell and interfering with essential metabolic processes by virtue of their chemical structure. Some of these agents, the antimetabolites proper, closely resemble in chemical configuration a normal metabolite essential for cell growth and proliferation. Such substances enter into the existing enzyme systems in place of the vitamin, amino acid or nucleotide precursor they resemble and block further developing reactions in the system. One obvious difficulty in the use of all these agents is that they are not selective. Chemotherapy of infections due to parasitic bacteria, rickettsiae or viruses is relatively more simple, at least in theory, since the microorganisms have biological systems and needs which differ substantially from those of the host cells, and antimetabolites capable of interfering destructively with the one need not affect the other. Moreover, the normal activity of the host defence mechanisms supplements the chemotherapeutic attack. The situation in neoplastic disorders in general and in the leukaemias in particular is quite different. Here, the biochemistry of the abnormal cells is not qualitatively grossly different from that of normal cells, and antimetabolites cannot be expected to exert a selective effect where it is required alone. They are potentially toxic to normal as well as to abnormal cells. Nevertheless, they are of considerable therapeutic value when carefully used since cell susceptibility to destruction by such agents appears generally to be closely related to mitotic activity, and rapidly proliferating leukaemic or malignant cells are more vulnerable than normal cells.

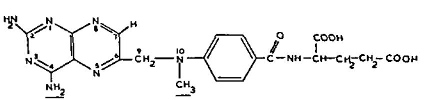
The steroid hormones are thought to affect cell proliferation by altering the environment so as to render it unfavourable to the development and multiplication of abnormal cells, particularly those derived from haemopoietic elements. In the same category may perhaps be placed certain factors of unknown nature present in normal blood or resulting

Lactobacillus casei and *Leuconostoc citrovorum* is strongly inhibited (Hendlin, Koditschek and Soars, 1953; Sauberlich, 1949); *in vitro* cultures of bone-marrow cells and fibroblasts show defective mitotic activity (Jacobson, 1952; Kieler and Kieler, 1954); embryonic growth and differentiation has been shown to be inhibited in several species (Goldsmith, Harnly and Tobias, 1950; Wagley and Morgan, 1948). In experimental animals a megaloblastic anaemia can be produced, followed by marrow aplasia (Thiersch and Philips, 1949). Degeneration of other rapidly proliferating tissues, in particular the intestinal tract, also



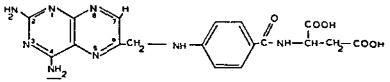
4-AMINOPTEROYLGLUTAMIC ACID (AMINOPTERIN)

FIG. 20.



4-AMINO-N[10] METHYL PTEROYLGLUTAMIC ACID (AMETHOPTERIN)

FIG. 21.



4-AMINOPTEROYLASPARTIC ACID (AMINO-AN-FOL)

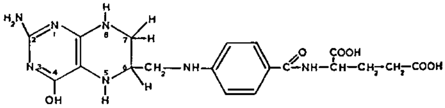
FIG. 22.

takes place. A relation between folic acid antagonists and the action of oestrogens has been noted by Hertz (1948) and Hertz and Tullner (1949), who found that the 4-amino derivatives of folic acid inhibited the stimulatory effect of oestrogens on the growth of the oviduct of young chicks and the uterus of immature rats.

Although the 4-amino analogues are the only antagonists of folic acid to find general clinical use at present, much fundamental work of considerable potential importance has been done on simpler substances with anti-folic activity. These include the 2:4-diamino-5-chlorophenyl pyrimidines (Hitchings, Falco, Vanderwerff, Russell and Elion, 1952), and

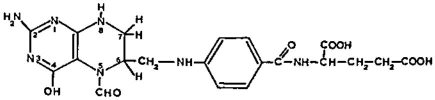
stages in nucleic acid synthesis which require folic acid derivatives are interrupted by suitably active folic acid antagonists.

Of the very large number of analogues of pteroylglutamic acid, three have shown particularly high activity as antimetabolites. These are all compounds in which the hydroxyl group in the 4-position in the folic acid molecule has been replaced by an amino group. They are: 4-aminopteroylglutamic acid (aminopterin), 4-amino-N^{10}-methylpteroylglutamic acid (amethopterin or methotrexate), and 4-aminopteroyl aspartic acid



5 6 7·8 TETRAHYDROPTEROYLGLUTAMIC ACID

FIG. 18.



5-FORMYL 5·6·7·8 TETRAHYDROPTEROYLGLUTAMIC ACID (FOLINIC ACID)

FIG. 19.

(amino-an-fol). The structural formulae of these three compounds are given below (Figs. 20, 21, 22).

The close similarity between the structure of these analogues and that of folic acid makes it highly probable that their mode of action is as specific antimetabolites, and indeed the signs and symptoms of experimental poisoning with these substances resemble those of folic acid deficiency. The antimetabolic effects cannot be satisfactorily counteracted or reversed by folic acid, even in high doses, unless the folic acid is given before the antagonist, so that a store of folinic acid is available (Goldin, Greenspan, Venditti and Schoenbach, 1952), but folinic acid is successful in this respect (Burchenal and Babcock, 1951), an observation lending strong support to the supposition that the analogues interfere with the system of hydrogenating and formylating enzymes which normally convert folic acid to its active folinic form.

Extensive studies of the effects of these 4-amino derivatives of folic acid on the growth of bacterial and animal tissues have been carried out. The growth of *Streptococcus faecalis*,

bone and joint pains, hepatomegaly, splenomegaly and lymphadenopathy, and the blood and marrow, though retaining leukaemic features, return towards more normal cytology. Remissions, whether complete or partial, are always temporary and recurrence of the full leukaemic state ensues after a period varying between a few weeks and occasionally as long as 2 years, with an average duration of about 8 months (Burchenal and Ellison, 1956).

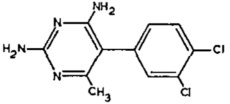
Folic acid antagonists are given orally in a single daily dose of 0·25 to 0·5 mgm. of aminopterin or 2·5 to 5·0 mgm. of amethopterin. The drugs can be given intramuscularly, but absorption is equally rapid from the intestinal tract. Experiments using amethopterin (Burchenal, Waring, Ellison and Reilly, 1951) have shown an appreciable level of the drug in serum within 15 to 30 minutes after an oral dose with rapid disappearance in from 3 to 12 hours. The blood-level remains high for much longer in patients with renal insufficiency and particular care must be taken in the management of such patients. Normally treatment is continued with daily doses of the chosen antagonist for 3 or 4 weeks until there is clear evidence of remission or until buccal ulceration, loss of hair, and other early signs of toxicity appear. When toxicity occurs, Burchenal (1954) recommends that treatment be stopped for 7 to 10 days and restarted, after subsidence of the toxic signs, at a lower dosage. It may be necessary to continue treatment for as long as 2 months before remission occurs, but in most responsive cases improvement is manifest within 2 to 3 weeks. Maintenance therapy may be continued throughout the remission, the dose being reduced by about half and given on alternate days, or intermittent courses at full dosage may be given whenever the blood or marrow shows evidence of deterioration. Whichever course is followed, eventually a refractory state develops and the disease can no longer be controlled by the same drug. Studies with transplantable animal leukaemias suggest that the leukaemic cell succeeds in modifying its metabolic processes sufficiently to become drug-resistant, since successive transfers from animals treated with folic acid antagonists give rise to transplants which show consecutive increase in resistance when the recipient animals are treated with the antimetabolite concerned (Law and Boyle, 1950).

The acquisition of resistance in mouse leukaemias treated by amethopterin is thought to result from the selective overgrowth of individual resistant cells produced by random mutation (Law, 1952). An important biochemical feature of resistant leukaemic cells is that, in the presence of the folic acid antagonist concerned, they are able to continue converting folic acid to citrovorum factor much more efficiently than are sensitive cells in similar circumstances (Nichol, 1954). The experiments of Jacobson (1954*a* and *b*) suggest that resistant tissues may be capable of converting aminopterin into an inactive form, but the nature and mechanism of this inactivation remain obscure.

The prevention or reversal of toxic effects of folic acid antagonists by citrovorum factor has been mentioned previously, and this substance may be used clinically to control severe toxicity, but the chemotherapeutic actions of the antimetabolites are also usually nullified by citrovorum factor, which may indeed exacerbate the leukaemic state, and in general this factor is contraindicated in acute leukaemia except when the patient's condition is critically endangered by antimetabolite toxicity (Burchenal, 1954). Nevertheless, occasional reports of an apparently synergistic action of aminopterin and citrovorum factor have been published (Consoli, 1955) and a short trial of combined therapy might be justified in patients particularly prone to aminopterin toxicity.

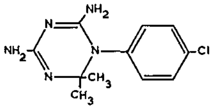
a series of dihydrotriazines (Modest, Foley, Pechet and Farber, 1952). Representative formulae are shown in Figs. 23 and 24. Clinical trials with these compounds have shown them to be less effective therapeutically and more toxic than amethopterin (Murphy, Ellison, Karnofsky and Burchenal, 1954; Farber, Toch, Sears and Pinkel, 1956).

The clinical application of the 4-amino antagonists of folic acid has been the subject of many reports since the value of these drugs in acute leukaemia was first established by Farber and his associates (1948). The therapeutic position has not altered significantly since comprehensive reviews were published by Dresner and White (1952) and in the Proceedings of the Second Conference on Folic Acid *Antagonists in the Treatment* of Leukaemia (1952). The drugs are relatively ineffective in adults, but are capable of



2 4-DIAMINO-5-DICHLOROPHENYL METHYL PYRIMIDINE

FIG. 23.



4-6-DIAMINO-1-CHLOROPHENYL-1-2-DIHYDROTRIAZINE

FIG. 24.

producing some degree of remission in between 40 and 70 per cent of acute leukaemias of childhood. Burchenal (1954) recorded good clinical and haematological remission after the use of aminopterin and amethopterin in 44 of 119 children and 1 of 36 adults with acute leukaemia, while a compilation of records from many centres (Farber, 1951) showed that 68 per cent of 425 children with this disease had been improved. Monocytic leukaemia appears generally to be refractory to these drugs, but lymphoblastic, myeloblastic and stem-cell leukaemias are often amenable to treatment. There are at present no recognized criteria for predicting with certainty the response in an individual case, nor for assessing its prospective duration when remission occurs. The extent of remission achieved varies widely. Some 30 per cent of acute childhood leukaemias exhibit a complete return to normal, with loss of all clinical signs and symptoms of disease and development of normal blood and bone-marrow pictures, while in other remitting cases there is regression of fever,

Clinical trials of 6-mercaptopurine in acute and chronic leukaemias, malignant reticulo-endothelial disorders, and metastasising neoplasms of various kinds have made it clear that this antimetabolite is of considerable value in acute leukaemia and in chronic granulocytic leukaemia. It is substantially ineffective in all other malignant conditions, including multiple myeloma, lymphadenoma and many sarcomatous and carcinomatous tumours in which it has been tried (Burchenal *et al.*, 1953; Hall, Richards, Willet and Feichtmeir, 1954) with the possible exception of disseminated reticulum-cell sarcoma in which some improvement may take place. The results of treatment in acute leukaemia of children are comparable to those achieved with folic acid antagonists, complete clinical and haematological remissions of varying duration being induced in about 30 per cent of cases, with partial remissions in a further 20 per cent. The compound has, however, proved capable of producing remissions in some children whose disease has resisted folic acid antagonists, and is, moreover, much more effective than these antagonists in the control of acute leukaemia in adults. Hall *et al.* (1954) treated 24 adult patients and found over 50 per cent remissions, Fountain (1955) obtained 3 complete remissions and 1 partial remission in a group of 7 adults, and Whitelaw *et al.* (1956) found 7 complete and 8 partial remissions among 29 adults, but the experience of other centres has been rather less promising. Thus Burchenal and his associates (1953) recorded only 1 complete and 3 partial remissions among 12 adults, while Hayhoe (1955) found 2 complete and 5 partial responses in a group of 15 adults. A statistical analysis of clinical data from some twenty-three centres where 6-mercaptopurine had been used in acute leukaemia up to 1954 showed that about one adult in seven had responded satisfactorily, whereas in children the comparable figure was one in three (Bross, 1954).

Patients with chronic granulocytic leukaemia have also shown some good responses to this drug, with occasional satisfactory remissions maintained by continuous therapy. In chronic lymphatic leukaemia, on the other hand, no evidence of response has been obtained.

6-Mercaptopurine is given orally and is readily absorbed from the gastro-intestinal tract. The usual method of administration is in a daily dosage of approximately 2·5 mgm. per kg., continued until the production of leukaemic cells in the marrow is almost completely suppressed. Wide individual variation is encountered in the rate of response to the drug, and Hyman and her associates (1957) recommend that doses as high as 6·6 mgm. per kg. daily should be given for the first 2 weeks to achieve remission with maximum rapidity, dosage being then reduced to a third of this level for maintenance therapy. Patients should not be regarded as resistant until treatment has been maintained without response for at least 3 weeks. Frequent blood counts are necessary during the initial course, and a sharp drop in leucocyte count should lead to reduction of dosage. Nevertheless, it is probably best to push treatment to the stage of definite leucopenia before discontinuing the drug. Following withdrawal at such a stage, normal haemopoiesis may be re-established over the succeeding few days. When complete or partial remission is achieved the treatment may be discontinued and further courses given only when there is evidence of recurrence of disease in blood or bone marrow, but the total extent of remission is probably greater if maintenance treatment is continued throughout the whole illness until drug resistance finally develops. One might perhaps expect resistance to be acquired more rapidly by a population of cells continuously exposed to the antimetabolite, but this does

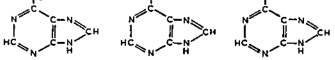
Folic acid antagonists have not proved of much value in chronic leukaemias or in other malignant reticulo-endothelial disorders.

Purine Antagonists

6-Mercaptopurine

Many synthetic analogues of naturally occurring purines and pyrimidines have been prepared by Hitchings and his associates in an extensive series of researches begun in 1942. These substances might be expected to act as antimetabolic antagonists in the early stages of nucleic acid synthesis in the body, and many of them have been subjected to clinical and experimental trials in a variety of neoplastic diseases, in the search for an agent which would influence neoplastic cell metabolism selectively without causing severe damage to normal cells. The most effective member of this group of analogues at present is 6-mercaptopurine, synthesized by Elion, Burgi and Hitchings (1952). Chemically this compound is closely related to adenine and hypoxanthine (Fig. 25).

Studies in the nucleic acid metabolism of *Lactobacillus casei* and other micro-organisms have shown that 6-mercaptopurine acts as a metabolic antagonist of adenine and hypoxanthine, and presumably the mode of action in mammalian tumours is similar, although



ADENINE HYPOXANTHINE 6-MERCAPTOPURINE

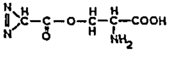
FIG. 25.

neither the chemotherapeutic nor the toxic effects can be reversed by purine administration. The studies of Hamilton and Elion (1954) in man, and of Elion, Bieber and Hitchings (1954) in mice, have shown that injected S^{35}-labelled 6-mercaptopurine may be incorporated into tissue nucleic acids and probably into the desoxyribonucleic acid of human leukaemic leucocytes.

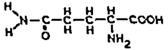
On the other hand, there is some evidence that interference with enzymes or coenzymes involved in growth processes may be of importance, and in particular the activity of coenzyme A, pantothenic acid and diphosphopyridine nucleotide may be affected (Biesele, 1954; Kaplan, Goldin, Humphreys, Ciotti and Stolzenbach, 1956). As Timmis (1957) points out, in coenzyme A adenine is linked via ribose and phosphoric acid to the pantothenic acid amide of beta-mercaptoethylamine, while diphosphopyridine nucleotide (DPN) also contains an adenine residue linked through ribose and phosphate to nicotinamide, so that an antimetabolic action of the adenine analogue 6-mercaptopurine against coenzyme A and DPN would not seem unreasonable.

The chief toxic effects of 6-mercaptopurine administration are exerted, like those of the folic acid antagonists, on cells of the bone marrow and haemopoietic tissues and the gastro-intestinal tract (Philips, Sternberg, Clarke and Hitchings, 1953). Hepatic degeneration may follow very high dosage (Clarke, Philips, Sternberg, Stock, Elion and Hitchings, 1953).

Melnick and Hartman, 1957). Unfortunately, azaserine possesses only slight anti-leukaemic activity in man when used alone, but since its site of action on the pathway of purine synthesis differs from that of 6-mercaptopurine, these two compounds have been used in combination. Burchenal and his associates (1955, 1956) used them together, each in a dose of 2·5 mgm. per kg. daily by mouth, and reported a very high rate of prolonged



AZASERINE



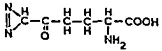
GLUTAMINE

FIG. 27.

remission in an admittedly specially selected group of patients. The enhancement of anti-leukaemic activity is paralleled by some increase in toxic effects, and the dosage of azaserine may need to be reduced if buccal ulceration becomes prominent. Enough reports have not yet been published for a clear impression to be gained of the superiority, if any, of the combination treatment over mercaptopurine alone when unselected series are compared, but the advantage is not likely to be great.

6-Diazo-5-oxo-*l*-norleucine (DON)

This antibiotic, isolated from Streptomyces, is similar in structure to azaserine (Fig. 28) and has a roughly comparable range of activity against experimental tumours *in vivo*.



DON.

FIG. 28.

Clinical observations by Magill and his associates (1957) showed a definite anti-tumour effect in patients with disseminated neoplasms, but in the few cases of leukaemia and lymphoma included in the trial the results were poor. Studies of DON activity in combination with purine analogues are being pursued.

Amino acid antagonists

White and Shimkin (1954) used ethionine, an analogue of the amino acid methionine, as an antimetabolic agent, but found it too toxic for clinical use. Hepatic damage,

not appear to be the case (Hyman, Brubaker and Sturgeon, 1957), at least so far as 6-mercaptopurine is concerned. During clinical use of 6-mercaptopurine in leukaemic states, toxic effects, apart from the marrow depressant action, are less prominent than those of folic acid antagonists. Mouth ulceration has been uncommon and gastro-intestinal symptoms rarely severe, although occasional patients experience anorexia, nausea, and vomiting during treatment and toxic hepatitis has been reported to occur after prolonged or heavy dosage (McIlvanie and MacCarthy, 1959). Rarely, drug fever may occur (Hyman, Gellhorn and Wolff, 1954).

Numerous other purine antagonists have been tested clinically after giving promise in experimental trials in leukaemic mice. They include purine itself, 6-chloropurine, 6-methyl-mercaptopurine, 2,6-diaminopurine, 6-thioguanine, and 8-azaguanine (Fig. 26). Apart

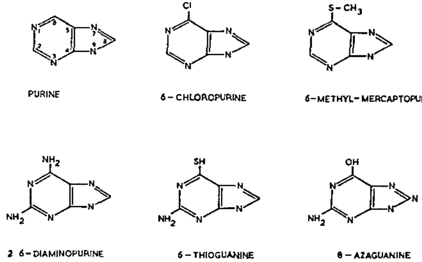


FIG. 26.

from 6-chloropurine and 6-thioguanine, which have activity roughly comparable to that of 6-mercaptopurine, these compounds proved less effective and more toxic than 6-mercaptopurine (Burchenal *et al.*, 1951, Gellhorn, 1953; Steinfeld *et al.*, 1954; Colsky *et al.*, 1955; Farber *et al.*, 1956; Ellison, Karnofsky and Burchenal, 1958). Since the development of resistance to one purine antagonist involves resistance to other members of the group, the alternative purine analogues have not found a place in clinical practice.

Other Antimetabolites

Azaserine

This compound, *o*-diazo acetyl-*l*-serine, was originally isolated from natural sources as an antibiotic and subsequently found to have considerable anti-tumour activity in experimental animals (Stock, Reilly, Buckley, Clarke and Rhoads, 1954; Burchenal, Murphy, Yuceoglu and Horsfall, 1954). Azaserine structurally resembles glutamine (Fig. 27) and inhibits purine biosynthesis by acting as a glutamine antagonist in the conversion of formyl-glycinamide ribotide to formyl-glycinamidine ribotide (Buchanan, Levenberg

abnormally utilized in leukaemia. This possibility has not been fully explored in leukaemic material, but there is some evidence (Van Vals, Bosch and Emmelot, 1956) that the hexose-monophosphate oxidative pathway is preponderantly used in a variety of solid tumours and perhaps also in mouse lymphomas (Kit, 1956), while Beck (1958) found some increased use of the pathway by leukaemic cells. Sahasrabudhe (1958) has therefore argued that, since the hexose-monophosphate pathway is not operative to a great extent in normal tissues, antimetabolic interference with the shunt might preferentially affect tumour growth without damaging normal cells. Analogues of 6-phosphogluconic acid have accordingly been prepared and used in experimental tumours with very encouraging preliminary results, but the work has not yet reached the stage of clinical trial.

The actinomycins

The mode of action of this group of antibiotics, derived from cultures of Streptomyces, is uncertain, but it appears likely that they have an antimetabolic effect, like that of the antibiotic azaserine. Actinomycins C and D have shown considerable anti-tumour activity in experimental animals, and have some clinical applicability in lymphomas and solid tumours, but they appear to be of little value in leukaemias (Farber *et al.*, 1956).

CYTOTOXIC DRUGS

Alkylating Agents

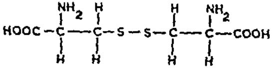
Nitrogen mustards

The cytotoxic action of mustard gas was observed during the First World War, when Krumbhaar and Krumbhaar (1919) noted leucopenia and marrow aplasia in cases of poisoning with this chemical-warfare agent. Various nitrogen analogues of sulphur mustard were synthesized by German and French chemists during the years between the First and Second World Wars, and from 1940 onwards many studies of biological activity of these compounds were carried out under conditions of wartime secrecy. After the end of the war a great deal of accumulated information was published by American, Canadian and British workers (Goodman *et al.*, 1946; Jacobson *et al*, 1946; Rhoads, 1946; Wilkinson and Fletcher, 1947; Wintrobe *et al.*, 1947; Craver, 1948; Gellhorn and Jones, 1949; Burchenal *et al.*, 1949; Brown and Davis, 1949, and many others) and the value of nitrogen mustards in the treatment of chronic leukaemia, Hodgkin's disease, and lymphosarcoma was clearly established.

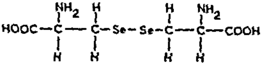
The mustard compounds owe their cytotoxic properties to the two chlorethyl groups, attached to sulphur in sulphur mustard and to nitrogen in the nitrogen mustards. The trivalency of nitrogen leaves a third bonding available, and variations in the nature of the substituent occupying this bond enable a homologous series of compounds to be synthesized. Those most commonly used in clinical work are tri-(2-chlorethyl) amine hydrochloride and di-(2-chlorethyl) methylamine hydrochloride. The structural formulae of sulphur mustard and these two nitrogen mustards are shown in Fig. 30. The nitrogen mustards in crystalline hydrochloride form are readily soluble in water but are then highly unstable, forming active quaternary ammonium compounds. They are therefore kept as dry crystals

lesions of the skin and gastro-intestinal tract, and psychotic states were readily produced.

Early and severe toxicity has also been encountered in the clinical use of selenium cystine, an analogue of cystine in which selenium replaces sulphur in the molecule (Fig. 29). Hepatic and renal damage did not occur, but nausea, vomiting, anorexia and diarrhoea were severe and there was marked loss of hair and damage to the nails (Weisberger and Suhrland, 1956). Remissions in acute leukaemia could, however, be produced, sometimes in patients who had become refractory to other antimetabolites. Although their high toxicity renders the available amino acid antagonists unsuitable for clinical use at present, they are of particular academic interest and potential importance because there is evidence that immature leukaemic leucocytes have a great avidity for certain amino



CYSTINE



SELENIUM CYSTINE

Fig. 29.

acids and may in consequence be highly susceptible to appropriate antimetabolic analogues (Weisberger, Suhrland and Seifter, 1956; Weisberger, 1957).

Pyrimidine antagonists

Metabolic antagonists of pyrimidines, and in particular of uracil, which may perhaps be utilized preferentially in nucleic acid biosynthesis in tumours, have been developed very recently and have shown convincing evidence of tumour-inhibiting activity. Hakala, Law and Welch (1956) studied 6-azauracil and some related compounds, and Heidelberger and his associates (1957) synthesized a variety of 5-fluorinated pyrimidines and reported high antimetabolic activity of 5-fluoro-uracil and 5-fluoro-orotic acid. Clinical trials of these compounds are at present being conducted and already give promise of valuable clinical activity in leukaemias and some solid tumours.

Antagonists of glycolysis

In Chapter 6 the differences between normal and leukaemic cell glycolytic metabolism were discussed, including the possibility that the hexose-monophosphate shunt might be

their activity predominantly against lymphocytic tissues and others against myeloid cells (Elson, 1955, 1958). The nitrogen mustards themselves show less specificity than do such derivatives as chlorambucil and the more remotely related methane sulphonyloxy alkanes, and for this reason they have a wider spectrum of action against leukaemias of different cytological varieties.

Chronic leukaemias, either myeloid or lymphatic, respond very well to intravenous administration of nitrogen mustards. Gardikas and Wilkinson (1951) and Wilkinson (1953) reported 74 per cent remissions in a series of 126 patients with chronic leukaemia treated in this way. Both haematological and clinical features of the disease are markedly affected and remissions so induced may last from a month or two to 2 or 3 years. Further treatment given as necessary may induce repeated remissions. Severe nausea and vomiting may occur during the course of the injections, but since recovery rapidly occurs, this does not provide a major contraindication to therapy. Like all other agents used in chronic leukaemia, the nitrogen mustards fail to alter the inevitable outcome of the disease, and response to repeated therapeutic courses becomes successively less complete and of shorter duration, until finally a refractory state is reached. It seems to have been the general experience that lymphatic leukaemia is less regularly amenable to treatment than myeloid, advanced cases in particular frequently failing to respond, but excellent remissions have been achieved in the earlier stages of the disease.

In acute leukaemia this group of drugs has proved quite ineffective, failing to alter the course of the disease in any significant manner. Although a decrease in the peripheral leucocyte count may be produced, the marrow picture, the physical signs and the clinical progress are uninfluenced.

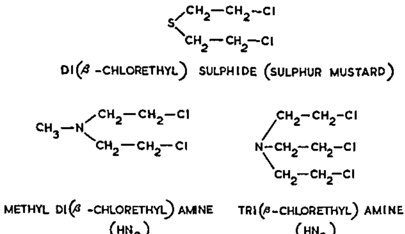
Occasional remissions in polycythaemia vera treated with these compounds have been reported (Spurr, 1950), but use in this disease does not seem to have been very extensive.

In all these conditions individual variation in response to treatment exists, and dosage must be regulated according to the day-to-day clinical and haematological findings. Each intravenous injection ordinarily contains 0·1 mgm. per kg. of body weight, dissolved in water immediately prior to injection. The dose may be repeated daily for 4 days in an average case, but some patients respond dramatically to a single injection, while others require six or eight before any substantial effect is observed. Dangerous thrombocytopenia and leucopenia may develop if a total dose exceeding 0·5 mg. per kg. is given over a short period of time. Some authorities therefore think it preferable, instead of giving a compressed course of several daily injections, to give a single dose and wait 5 or 6 days to assess the effect before repeating the dose. This procedure, though probably safer, has the disadvantages that more prolonged stay in hospital is needed and that the patient may suffer repeated attacks of nausea spread over perhaps a month, rather than condensing this unpleasant experience into a short span.

Chlorambucil

A series of water-soluble aromatic nitrogen mustards synthesized by Everett, Roberts and Ross (1953) included *p*-(di-2-chloroethylamino)-phenyl butyric acid (C.B.1348, Chlorambucil) (Fig. 31). This compound proved to be strongly inhibitory to tumour growth in animals, and clinical trials in man showed it to be an effective agent in the treatment of

and dissolved immediately before intravenous injection. The mechanism of the cytotoxic action of these compounds is not certain. They are highly active alkylating agents capable of combining readily with many biological substances, including certain enzyme prosthetic groups, but the concentrations achieved *in vivo* do not approach those necessary for enzyme inhibition *in vitro* and this property does not therefore offer an acceptable explanation of their activity. Studies of virus inhibition by nitrogen mustards show that inhibition is most marked in viruses containing predominantly desoxyribose nucleic acid, and *in vitro* these compounds have been demonstrated to react with phosphoryl, carboxyl and amino groups of nucleic acid. These and other studies of nucleic acid synthesis in the presence of nitrogen mustards suggest a direct action on desoxyribose nucleotides leading to mitotic inhibition (Goldthwait, 1952). *In vitro* studies of the interaction of nitrogen mustards and other alkylating agents with macromolecules, reported by Stacey and his



NITROGEN MUSTARD.

FIG 30.

associates (1958), strongly suggest that the most characteristic reactions under physiological conditions, when there is a great excess of sites available for alkylation, involve esterification of carboxyl groups in proteins and of phosphate groups in DNA. Bifunctional alkylating agents, with a pair of reactive groupings such as the two beta-chlorethyl groups of nitrogen mustard, appeared able to produce cross-links within different groups in a protein or DNA molecule, or between neighbouring molecules, thus altering the character of the molecules and preventing them from functioning normally. Whatever the precise mode of action, these drugs attack proliferating cells of most germinal tissues, in particular bone marrow, lymph glands and intestinal epithelium. Cell division is prevented at some premitotic stage and the more differentiated cells rapidly undergo karyorrhexis and disintegration. *Primitive, undifferentiated cells cease to proliferate,* but do not so readily undergo destruction. These biological actions of the nitrogen mustards and those of other alkylating agents are obviously similar to the actions of ionizing radiations, but the "radiomimetic" drugs are more selective in their haematological effects, some exerting

earlier nitrogen mustards can be seen from the structural formulae in Fig. 32. References to the development and trial of these derivatives may be found in the review article of Scott (1958).

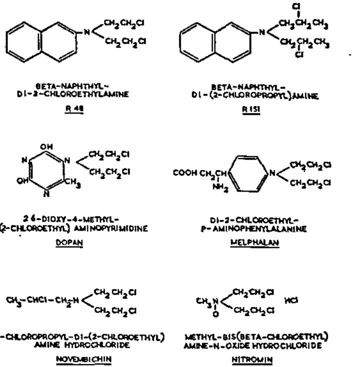


FIG. 32.

Triethylene melamine

Among a number of compounds other than 2-chlorethyl amines which are transformed *in vitro* into ethylenimonium derivatives, triethylenemelamine (TEM) has proved most

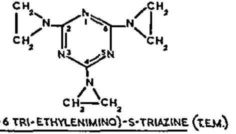
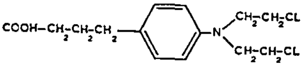


FIG. 33.

generally useful in neoplastic states. The structure of this compound, 2,4,6-tri-(ethylenimino)-S-triazine, is shown in Fig. 33. Reactive quaternary ethylenimonium compounds, with chemical and biological properties like those of the nitrogen mustard transformation

malignant lymphoma. Galton, Israels, Nabarro and Till (1955) found striking remissions in 4 of 23 patients with Hodgkin's disease, in 7 of 12 with lymphocytic lymphoma, in 4 of 8 with chronic lymphocytic leukaemia, and in 5 of 6 patients with follicular lymphoma. Other workers have had similar experiences in the use of the drug (Altman, Haut, Cartwright and Wintrobe, 1956; Bouroncle, Doan, Wiseman and Frajola, 1956; Ultmann, Hyman and Gellhorn, 1956). Reports from several centres presented in New York at a conference on "Comparative clinical and biological effects of alkylating agents" in March 1957 (*Ann. N.Y. Acad. Sci.*, 1958, Vol. 68, pp. 657–1266) gave brief details of the results of chlorambucil treatment in over 300 patients with lymphomatous disorders. Good responses were achieved in from 50 to 80 per cent of patients with chronic lymphatic leukaemia, and in an even higher proportion of patients with follicular lymphoma. Results in Hodgkin's disease were less satisfactory, but about 20–30 per cent of patients with the disorder manifested good remissions for 4 months or more, and many more showed some temporary benefit. The drug has proved relatively free from toxic side-effects and has the great advantage over TEM of being much less erratic in action and less likely to produce



DI-(β-CHLORETHYL) AMINOPHENYLBUTYRIC ACID
(CHLORAMBUCIL CB1348)

FIG. 31.

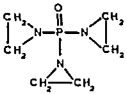
thrombocytopenia or marrow aplasia. Chlorambucil may therefore be particularly valuable in patients with chronic lymphatic luekaemia who have also moderate thrombocytopenia (Ultmann, Hyman and Gellhorn, 1956).

Chlorambucil is usually given by mouth in a daily dose of 0·2 mgm. per kg., but Galton and his associates (1955) recommend that this dose should be halved if there is lymphocytic infiltration of the bone marrow or when marrow hypoplasia is present. The initial dosage is continued, with weekly or fortnightly blood counts, until a satisfactory clinical and haematological response occurs. Treatment should not be regarded as ineffective until a trial of 4 weeks has been completed. Maintenance dosage adjusted to keep the remission as full as possible may be given, or treatment may be stopped until clear evidence of relapse indicates the need for a further course. It is not yet clear which of these methods of control is likely to give the more satisfactory results.

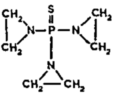
Other nitrogen mustards

Many other 2-chlorethyl analogues of the original nitrogen mustards have been synthesized, and several have been given clinical trials. None has yet found a place in clinical practice in the treatment of leukaemias, although some have proved valuable in lymphomas and solid tumours. These compounds include R48, R151, novoembichin, dopan, sarcolysine or melphalan, *nitromin*, and hemisulphur mustard. Their relationship to the

resembling TEM (Fig. 34), and possess an anti-tumour activity like that of the nitrogen mustards. They have been extensively investigated in proliferative diseases (Farber *et al.*, 1953; Sykes *et al.*, 1953; Shay *et al.*, 1953; Leonard, Israels and Wilkinson, 1956). These drugs are inclined to produce severe haemopoietic depression, although they do not have



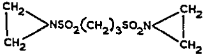
TRIETHYLENEPHOSPHORAMIDE (T.E.P.A)



TRIETHYLENE THIOPHOSPHORAMIDE (THIO-T.E.P.A)

FIG. 34.

the disadvantage of causing nausea and vomiting on parenteral administration. Either compound may prove effective in chronic granulocytic leukaemia, but the results in general are inferior to those obtained with other agents and the use of these compounds in leukaemias and allied states has been largely discontinued.



1·3-BIS(ETHYLENEIMINOSULPHONYL) PROPANE

(B.E.P.)

FIG. 35.

Two further related compounds, BEP (Fig. 35) and ethylenimino-benzoquinone, have been reported to induce remissions in chronic leukaemias (Paterson and Kunkler, 1954; Bernard, Mathé and Weil, 1957), but they do not appear to be as effective as chlorambucil or busulphan, and have not been widely used.

Busulphan

Busulphan (Myleran, 1:4-dimethanesulphonyloxybutane) is a sulphonic acid ester (Fig. 36) which, like the nitrogen mustards, is an active alkylating agent. Haddow and Timmis

products, are formed in an acid medium. The effects of TEM *in vivo* appear to parallel very closely those of the nitrogen mustards, producing cytotoxic changes of varying severity in proliferating tissues, particularly haemopoietic and lymphoid tissues. The substance is inexpensive, effective after oral administration, and does not produce nausea and vomiting of a degree comparable to that after nitrogen mustard injections.

Temporary remissions, often dramatic in nature, have been reported in many patients with Hodgkin's disease, lymphosarcoma, chronic myeloid and lymphatic leukaemia and in some cases of polycythaemia vera (Karnofsky *et al.*, 1951; Paterson and Boland, 1951; Rundles and Barton, 1952; Gellhorn, Klingerman and Jaffe, 1952; Silverberg and Dameshek, 1952; Kravitz, Diamond and Craver, 1952; Rosenthal and Rosenthal, 1952; Paterson and Kunkler, 1953; Wasserman, 1954). The therapeutic effect is most marked in Hodgkin's disease, chronic lymphocytic leukaemia, and other generalized lymphomatous processes. In granulocytic leukaemia results are less good, and in acute leukaemias and multiple myeloma TEM appears ineffective.

The major problem in the application of TEM to treatment is the wide individual variation in susceptibility to its action. Although a relationship between type of disease and sensitivity is not clear-cut, it seems that a response to small doses is especially common in chronic lymphatic leukaemia. Certainly the variation in tolerance is very great; evidence of toxic depression of the bone marrow with thrombocytopenia, leucopenia and aplastic anaemia may develop after as little as 8 to 10 mgm. of TEM given over a period of 7 days, while some patients will tolerate 10 to 15 mgm. weekly for long periods. Some of these wide discrepancies in dosage may be due to differences in gastric acidity, since the drug breaks down in an acid medium. Attempts have been made to counteract this effect by giving the drug an hour before breakfast together with a suitable alkali (2 gm. sodium bicarbonate) to facilitate absorption, but response remains poorly predictable.

In clinical use it is therefore wise to start treatment with small doses of the order of 2 to 3 mgm. daily for 2 or 3 days, and to give no further treatment for at least 10 days when the extent of bone-marrow depression produced by the initial course can be assessed. Patients sometimes manifest an increasing sensitivity to toxic action of repeated courses of TEM, but in general the individual reaction to a given dose remains fairly constant as far as marrow depressant effect is concerned. The average total dose of TEM needed to provoke substantial remission in responsive cases of lymphadenoma or lymphatic leukaemia is about 20 mgm. Throughout the course, careful studies of the peripheral blood should be made at frequent intervals, particular attention being paid to the leucocyte count and to the platelet level. Further doses of TEM should not be given to a patient whose leucocyte or platelet count is falling abruptly, but should be withheld at least until the blood picture has stabilized and preferably until initially normal constituents have returned to normal limits. When a remission has been satisfactorily induced by TEM, it is generally thought best to withhold the drug until there is clear evidence of relapse. Provided these precautions are taken and the powerfully toxic nature of the drug appreciated, it can be used without undue risk.

TEPA and Thio-TEPA

These two ethylenimine derivatives of phosphoric acid, triethylene phosphoramide (TEPA) and triethylene thiophosphoramide (thio-TEPA), have a structure somewhat

Busulphan may be given in successive courses or continuous maintenance treatment may be used. Initial treatment consists usually of from 4 to 10 mgm. daily, by mouth, continued under careful haematological control until the granulocyte count falls to about 20,000 per cu. mm. Thereafter treatment may be discontinued, to be recommenced when relapse takes place, or maintained at a level adjusted to keep the leucocyte count between 10,000 and 20,000 per cu. mm. Maintenance dosage is usually from 1 to 2 mgm. daily, but higher doses are occasionally necessary. After a variable period of remission every patient eventually becomes resistant to busulphan, but resistance does not appear to develop earlier with maintenance treatment than with intermittent courses (Galton, Till and Wiltshaw, 1958), and since a more stable control can often be achieved by continuous therapy, this method of management is generally preferred (Bethell, 1958).

In chronic lymphocytic leukaemia, as in Hodgkin's disease and lymphosarcoma, busulphan is sometimes effective in reducing the glandular enlargement and improving the leucocyte picture (Sykes 1958), but the doses required are large and there is a serious danger of thrombocytopenia and marrow aplasia being induced. The drug has been of no value in acute leukaemias, nor in most cases of myeloblastic transformation in chronic granulocytic leukaemia, although Di Pietro and Gallico (1955) recorded a remission induced by busulphan in one such case.

Other Cytotoxic Drugs

Desacetylmethylcolchicine

Colchicine has long been known to inhibit cell mitosis, and the potentialities of this substance in the treatment of proliferative conditions such as leukaemia and malignant neoplasms have been recognized and explored (Dustin, 1938; Bucher, 1939). The material proved, unfortunately, to be far too toxic for clinical use. The isolation by Santavy and Reichstein (1950) of a new alkaloid, desacetylmethylcolchicine, from *Colchicum autumnale*, appears to have provided a partial solution to this difficulty. The new alkaloid possesses strong mitosis-inhibiting activity, but is very much less toxic than colchicine.

Extensive clinical trials have recently been taking place, and reports of several series of cases have appeared since 1953 (Bock and Gross, 1953, 1954; Moeschlin, Meyer and Lichtman, 1953, 1954; Storti and Gallinelli, 1954; Leonard and Wilkinson, 1955; Gigante, 1955; Keibl, 1955; Moeschlin, 1957) from which a preliminary assessment of the value of desacetylmethylcolchicine (demecolcine) can be made. The drug may bring about marked improvement in both haematological and clinical features of chronic myeloid leukaemia; good remissions have been achieved in over 90 per cent of the cases described by the authors cited above. A more specific granulocyte depressing action is exerted by demecolcine than by most other chemotherapeutic agents used in chronic myeloid leukaemia, and there is little tendency for thrombocytopenia or aplastic anaemia to complicate treatment. Results in the few cases of acute leukaemia in which demecolcine has been used have been less encouraging, but some partial remissions have been induced. In other malignant reticuloses and in various tumours results have been unsatisfactory, although Storti and Gallinelli (1954) found some improvement to result from long-term treatment in Hodgkin's disease.

Demecolcine is given orally in a dosage varying between 1 and 10 mgm. daily. In chronic myeloid leukaemia it is customary to initiate treatment with 1 to 3 mgm. daily,

(1953) found this compound intensely inhibitory to certain animal tumours and noted a strong depressant effect on the myeloid cells in a dosage that did not significantly affect haemopoietic cells of other series. Studies conducted in the clinical field have shown that busulphan is a most valuable controlling agent in chronic myeloid leukaemia, and reports from many centres reveal a high percentage of good initial remissions, some in patients who had become refractory to X-ray treatment (Galton, 1953, 1956; Bollag, 1953; Wilkinson, 1953; Hansen, 1954; Wagner, 1954; Haut, Altman, Cartwright and Wintrobe, 1955; Bernard, Mathé and Najean, 1955; Galton and Till, 1955; Kurrle, 1955; Louis, Limarzi and Best, 1956; Blackburn, King and Swan, 1956; Frost and Jackson, 1956; Greig, 1956; Hyman and Gellhorn, 1956).

Pharmacologically, the cytotoxic action of busulphan is of particular interest, since it appears to be limited essentially to the bone marrow. Cells of lymphoid germinal tissues and of the gastro-intestinal tract, commonly affected by cytotoxic agents in parallel with the haemopoietic cells, are not appreciably damaged by busulphan. In low doses a selective inhibitory effect on granulocyte precursors is obtained, although thrombocyte formation is also depressed by relatively small doses. The drug is readily absorbed from the gastro-intestinal tract but its fate in the body and the precise mode of action are not

$$CH_3SO_2{-}O{-}CH_2{-}CH_2{-}CH_2{-}CH_2{-}O{-}SO_2CH_3$$

1 4-DIMETHANESULPHONYLOXYBUTANE

(MYLERAN, BUSULPHAN)

FIG. 36.

yet determined, although studies with P^{32} have shown that the formation of new DNA is depressed during administration (Li, Leonard and Harrison, 1956).

Clinically, in chronic granulocytic leukaemia almost all patients respond initially to busulphan, with symptomatic improvement, reduction in splenomegaly and hepatomegaly, fall in leucocyte count, reticulocytosis and increase in haemoglobin. Thrombocytopenia may develop after the leucocyte count has become stabilized at a low level for some weeks, and this appears to be the only important side-effect likely to be encountered at all commonly. Myeloid aplasia may be produced by excessive dosage (Wilkinson, 1953; Galton and Till, 1955) and has been reported in patients having low-dosage maintenance treatment (Storti and Pederzini, 1955; Hayhoe and Kok, 1957), but with increasing experience in the use of busulphan the risk has become very small. A second rare complication which may arise during treatment is renal failure due to hyperuricaemia following very rapid breakdown of granulocytes. This can be avoided by adjusting dosage to prevent too sharp a fall in the leucocyte count and by ensuring a high fluid intake (Hyman and Gellhorn, 1956).

Less important side-effects observed in patients receiving busulphan include skin pigmentation and amenorrhoea, but in general busulphan is singularly free from unpleasant side-actions. Remissions lasting from a few months to 4 years have been recorded (Galton, Till and Wiltshaw, 1958), but the drug has not yet been in use long enough to assess its full potentialities.

2-hydroxystilbamidine, the essential disease process does not seem to be radically affected and the hyperglobulinaemia and Bence-Jones proteinuria are not altered. The diamidines are given intravenously in daily doses of 100 to 150 mgm. dissolved in 200 ml. of 5 per cent glucose in distilled water, a course consisting of 15 to 20 such injections. These injections require particular care since the material is irritant and readily produces obliteration of veins, and this technical disadvantage has also tended to discourage the use of diamidines in myeloma.

Urethane

Urethane (ethyl carbamate) has long been recognized to be a protoplasmic and mitotic poison (Warburg, 1910), but its use in neoplastic disease dates from the studies of Haddow and Sexton (1946). Carcinostatic activity in experimental cancers was marked and trials in patients with extensive carcinomatosis were conducted. Although the results were disappointing, the occurrence of leucopenia in treated patients led to further studies of its action in leukaemia. Paterson, Haddow, Ap Thomas and Watkinson (1946) and subsequently many investigators found the drug effective in chronic leukaemias, both granulocytic and to a less extent lymphocytic. It was almost entirely valueless in acute leukaemias, but produced favourable results in many cases of multiple myelomatosis.

Urethane inhibits cell division in germinal tissues such as bone marrow and gastro-intestinal epithelium, perhaps by interfering with a hypothetical phosphokinase, required for normal nucleic acid metabolism (Boyland and Rhoden, 1949), but isotopically labelled urethane is not apparently selectively localized in rapidly dividing cells or in cellular nucleic acids (Bryan, Skipper and White, 1949). The drug is easily absorbed from the gastro-intestinal tract and rapidly broken down with the formation of carbon dioxide, ethyl alcohol, and ammonia, and only about 1 per cent of the reactive carbonyl grouping is retained and distributed about the body tissues according to Boyland and Rhoden (1949) and Bryan *et al.* (1949). The major toxic effects of urethane, apart from the haemopoietic depression, are anorexia, nausea and vomiting, and despite the use of enteric-coated capsules these symptoms may occasionally prevent the continuance of treatment.

Clinically the chief use of urethane has undoubtedly been in the management of chronic granulocytic leukaemia. The usual dose is 0·5 to 1 gm. three times daily, given by mouth as an elixir or in enteric-coated capsules. The course is continued for from 2 to 6 weeks until the leucocyte count has been reduced to about 50,000 per cu. mm., and the dose is then decreased to about 1 gm. daily. Maintenance treatment of 0·5 to 1 gm. daily is advisable. Most patients react satisfactorily, but careful haematological supervision is necessary during the initial weeks of treatment since thrombocytopenia, agranulocytosis and aplastic anaemia may be produced by overdosage in a sensitive individual and the marrow depressant effects continue for some time after cessation of treatment. Accompanying the leucocyte fall in responsive patients a rise in haemoglobin level takes place together with regression of spleen and lymph nodes and subjective improvement.

In chronic lymphatic leukaemia results have generally been less satisfactory. The high leucocyte count may be greatly reduced, but regression of lymph nodes and other tissue infiltrations is not usually marked and subjective improvement is not prominent.

increased after 3 or 4 days to 5 to 10 mgm. daily and maintained at this level until the leucocyte count drops to between 20,000 and 30,000 per cu. mm. This may take from 3 to 6 weeks to achieve. Treatment is then stopped temporarily for 3 or 4 days until the leucocyte level ceases to fall, and maintenance dosage is then recommenced with from 1 to 5 mgm. per day. Frequent blood counts are necessary during the earlier stages of treatment, since there is considerable individual variation in response, but once a satisfactory remission is attained the interval between counts may be increased to 3 or 4 weeks. Continuous maintenance therapy has been found more satisfactory than intermittent courses. *Toxic effects with demecolcine have not been striking, although nausea is sometimes* experienced on high doses and occasionally mild pruritus and loss of hair may occur. Moeschlin (1957) has emphasized that the drug may be useful in some cases of terminal myeloid blast cell transformation, but that it is strictly contraindicated in lymphatic leukaemia since it may exacerbate the disease.

Arsenic

Inorganic arsenic depresses leucopoiesis and has been used for many years in the treatment of chronic myelocytic leukaemia. Many patients with this disease may derive considerable benefit from continued treatment with arsenic, usually given as Fowler's solution in doses of 0·3 to 0·5 ml. three times daily. A state of mild chronic arsenic poisoning must be induced to achieve a satisfactory therapeutic level, and because of this drawback and the existence of several more effective and less troublesome chemotherapeutic agents, not to mention radiotherapeutic methods of control, arsenic is little used in leukaemia at the present time.

Diamidines

Stilbamidine and pentamidine have been therapeutically successful in kala azar, and since the marked hyperglobulinaemia of this disease is paralleled by a similar globulin increase in multiple myeloma, the possibility that these drugs might be valuable in the latter disease was considered. Moreover, the cytoplasmic basophilia and pyroninophilia of myeloma cells indicates the presence of large amounts of ribose nucleic acid, and Kopac (1945) has suggested that such nucleoproteins may be denatured and precipitated by diamidines. Furthermore, ribose nucleic acid inhibits the antibacterial action of stilbamidine and it seems probable that the cationic diamidines block essential metabolic processes *by combining with nucleic acids* (Bichowsky-Slomnitzky, 1948). Clinical trials undertaken at many centres revealed that significant benefit ensued in roughly half the cases, but dramatic remissions were uncommon (Snapper, Turner and Moscowitz, 1953). Stilbamidine has a number of side-effects which militate strongly against its widespread use. The most important of these is a dissociated anaesthesia of the trigeminal area with loss of touch sensation but retention of pain and temperature sense. There are also unpleasant tingling and burning paraesthesiae which may persist for months after termination of treatment. In addition, the drug exacerbates the defects of renal function so commonly present in multiple myeloma. Another diamidine derivative, 2-hydroxystilbamidine, is more satisfactory in these respects and apparently causes neither trigeminal neuropathy nor impairment of renal function (Snapper, 1948). Although amelioration of clinical symptoms, in particular bone pains, is often brought about by either stilbamidine or

however, 30 enjoyed complete remissions and 19 partial improvement. In many reports it is not possible to distinguish adult from childhood cases, but where the age has been given, there seems no doubt that children responded very much better than adults. Fessas *et al.* recorded complete remissions in 18 of 22 children with lymphoblastic leukaemia as compared with 3 out of 9 in older patients. They encountered no complete remissions in 15 cases of acute myeloblastic leukaemia nor in 1 acute monocytic leukaemia, and indeed found evidence of an acceleration of the leukaemic process by cortical steroids in some of these cases. The vastly better results achieved at most centres in treating lymphoblastic and undifferentiated leukaemias as compared with myeloblastic and acute monocytic forms of the disease suggests that the lymphoid antagonistic action of the hormones may be the operative factor in inducing remissions. Relapse invariably takes place, however complete the remission, and although second and even third remissions may be induced by repeated therapy a refractory state is eventually reached.

There is little to choose between corticotrophin and cortisone in the control of acute leukaemia, although the first must be injected while the second may be taken by mouth. Customary dosage in leukaemia is from 60 to 150 units of ACTH or 100 to 400 mgm. of cortisone daily in divided doses. Early signs of hypercorticism, with weight increase and fluid retention, are usually produced by such treatment, but serious toxic signs of overdosage are not common in leukaemic patients, who appear to have a remarkable tolerance to these hormones. The synthetic steroids prednisone (metacorten) and prednisolone, introduced in 1955 (Bollet *et al.*, 1955; Dordick and Gluck, 1955; Margolis *et al.*, 1955), are closely related in structure to cortisone, but have increased potency with minimal salt-retaining properties. Their effects in leukaemia appear to be very comparable to those of ACTH and cortisone and the relative freedom from salt and water retention when they are used has led most clinicians to prefer them, especially when long-term or high-dosage treatment is envisaged. The anti-leukaemic activity of prednisone and prednisolone is from 2 to 5 times that of cortisone and a proportionate reduction in dosage is therefore made, with initial doses of 50 to 150 mgm. Whichever steroid is used, continued maintenance treatment at a decreased dosage may be given after remission has been induced, and this is probably the best course of action when the improvement is partial. When complete remission occurs, with return to normal of the peripheral blood and marrow and disappearance of leukaemic physical signs and symptoms, the treatment may be withdrawn until signs of relapse appear, in the hope that the inevitable development of a resistant state may be postponed. Although such remissions commonly last only a few weeks, occasional cases remain in full remission for many months. Since infections are frequent in leukaemic patients and liable to be exacerbated by steroid therapy, antibiotics are usually given concurrently with the steroids. It is to be noted that the beneficial effects of steroid hormonal therapy usually appear much more rapidly than do those of the antimetabolites, and clear evidence of commencing remission may be present within a few days. Nevertheless, occasional patients are slow to respond and treatment should not be discarded as ineffective until a trial has lasted 2 to 3 weeks. The use of steroids in combination or alternation with antimetabolites in acute leukaemia is discussed in Chapter 12.

Attempts have recently been made to influence the course of myeloblastic leukaemias and chronic myeloid leukaemias in a terminal myeloblastic phase by massive doses of

The effect of urethane in multiple myeloma has been the subject of rather conflicting reports. Earlier observations by Alwall (1947), Loge and Rundles (1949), and Rundles, Dillon and Dillon (1950) gave rise to considerable optimism, since over 50 per cent of their cases experienced reasonable improvement, with diminution of fever and bone pains, reduction in marrow myeloma cells, X-ray evidence of healing of bone lesions, and in some cases sharp reduction in the abnormal serum proteins. Later reports have been discouraging. Luttgens and Bayrd (1951) treated 66 cases and found objective improvement in only 20 per cent, while even these cases showed no change in bone marrow, serum proteins or Bence-Jones proteinuria. Snapper and his associates (1953) could obtain a favourable response to urethane in only 3 of 25 cases and found nausea and vomiting after oral and even after subcutaneous administration of the drug to be of prohibitive severity in a very high proportion of their cases. The dosage necessary to obtain improvement is high, from 2 to 5 gm. daily continued until a total quantity of 240 to 300 gm. has been given. Favourable results are more likely to be achieved in the early stages of the disease.

HORMONAL AGENTS

Corticotrophin (ACTH), cortisone and related compounds

The mode of action of corticotrophin and the adrenal cortical steroids in diseases of the blood and reticulo-endothelial system has not been clearly elucidated, despite intensive studies during the last decade. The work of Dougherty and White (1944, 1947) showed that these hormones caused disintegration of lymphocytes in experimental animals and that a reduction in lymphoid tissue was brought about and maintained during the period of excessive adrenocortical steroid activity. Lymphocytopenia following ACTH or cortisone in man appears to be a less constant or sustained feature (Forsham *et al.*, 1948; Sprague *et al.*, 1950), but there is an initial lymphocytolytic effect. Observations of this kind stimulated clinical research into the value of these steroid hormones in proliferative diseases of the haemopoietic and lymphoid system, and as a result of trials at many centres it soon became clear that their usefulness was substantially restricted to the acute leukaemias. In other reticulo-endothelial diseases, including malignant lymphomata and some chronic leukaemias, a secondary element of haemolytic anaemia or thrombocytopenic purpura may be present, and in these cases the steroid hormones may be of benefit by controlling the haemolytic or purpuric manifestations, but the essential disease process is not greatly influenced.

Since the early observations of Pearson *et al.* (1950) and Farber *et al.* (1950) the effect of the steroid hormones in acute leukaemia has been the subject of numerous reports. Remissions of good clinical and haematological extent in 80 out of 175 children and 14 out of 60 adults with acute leukaemia were recorded in the Proceedings of the Second Clinical ACTH Conference (1951). The M.R.C. Panel reports (1952, 1953) in Great Britain were less promising, only 5 out of 24 cases showing a response. Fessas *et al.* (1954) collected data from 26 reports referring in all to 425 cases of acute leukaemia. Among 217 cases of acute undifferentiated leukaemia, 88 experienced complete remission, 68 partial remissions and 61 failed to respond. Only 1 complete and 1 partial remission were recorded out of 26 acute monocytic leukaemias, and only 4 complete and 13 partial remissions among 72 myeloblastic leukaemias. Out of 110 lymphoblastic leukaemias,

some kind of specific anti-leukaemic effect may be exercised by such transfusion. Dreyfus (1948) discovered 22 authentic examples of remission in acute leukaemia recorded in the literature prior to that time, and in 19 of these, blood transfusions had been given, whereas no other therapeutic agent had been constantly used. Bierman *et al.* (1950) found that the use of blood transfusions and antibiotics increased the average survival of acute leukaemic patients from 5·6 to 8·9 months, although Southam *et al.* (1951) did not think these measures influenced the remission rate. Bessis and Dausset (1950) reported the results of exsanguino-transfusion in 60 cases, of whom some 20 per cent experienced a more or less complete remission, while Hayhoe and Whitby (1955) found 14 remissions, 5 of them complete, in 41 patients treated initially with whole blood transfusion alone. These investigators agree that blood transfusion may provoke remission in acute leukaemia of any cytological kind, although lymphoblastic cases are more likely to respond than either myeloblastic or monoblastic. The nature of the blood factor responsible for the induction of these remissions is unknown, and no particular blood appears to have greater therapeutic efficiency than others.

An awareness of the possible specific therapeutic value of blood transfusion is particularly necessary, not only because of the intrinsic merits of such treatment, but because transfusions are so often used as supportive measures during the course of treatment with chemotherapeutic agents that clinical trials of the effects of such agents may be complicated by the difficulty of distinguishing remissions due to the agent under trial from those attributable to the transfused blood.

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steroid hormones. Complete clinical and haematological remissions were induced in some cases by from 3 to 5 gm. of cortisone in a single injection, or 1 to 3 gm. daily by mouth for 2 to 4 days (Bernard and Deltour, 1953; Bernard *et al.*, 1954). Secondary effects in this series of patients were apparently not marked or dangerous despite the huge dosage employed. Similar studies have been reported in which 9-alpha-fluoro-hydrocortisone, a halogenated derivative of hydrocortisone, was used. Biological studies of this compound, which was synthesized initially by Fried and Sabo (1953), showed that its activity was between 10 and 25 times that of cortisone as regards glycogenesis, sodium and potassium regulation and other metabolic effects (Liddle *et al.*, 1954). Hill and Vincent (1955) used this material in the treatment of 16 cases of acute leukaemia. Huge daily doses from 250–400 mgm., theoretically equivalent to 5 to 10 gm. of hydrocortisone, were employed without any serious toxic effects. Dietary sodium restriction and added potassium by mouth and intravenously kept the serum level of these substances within normal limits, but some hypertension, acneiform eruptions, and mental disorders were encountered. Complete remissions, both clinical and haematological, occurred in 4 out of 9 cases of acute myeloid leukaemia and 2 out of 4 cases of lymphoblastic leukaemia. Several partial remissions also occurred. Improvement was very rapid but relapse occurred within 6 weeks. The authors noted that there was evidence of increased erythropoietic activity, with peripheral erythroblastosis in several cases, while in others the rapidity of the platelet increase suggested a direct stimulatory effect.

Ranney and Gellhorn (1957) gave massive doses of prednisone, totalling 1 gm. daily for 10 days, to 18 patients with acute leukaemia. Complete remissions lasting from a few weeks to several months ensued in 5 cases and partial remissions of similar duration in 6. Nearly all the patients in this series were adults. The morphological classification of the cases proved difficult and no general conclusion could be drawn about the relationship of the variety of acute leukaemia and the response to therapy, although the authors noted that most of the complete remissions occurred in patients whose blood-smears were rather more compatible with lymphoblastic than with myeloblastic leukaemia. In these patients and in further groups with subacute leukaemia or lymphoma given similar treatment with only poor response, toxic effects were considerable and included hyperglycaemia, pyogenic infections, bacteraemia and septicaemia, generalized fungus infection, psychoses, and gastro-intestinal ulceration and perforation. In view of the serious and frequent side-effects and the short duration of the remissions induced, Ranney and Gellhorn did not think it reasonable to recommend massive prednisone dosage as a treatment of choice in acute leukaemia in adults, and even less so in children, whose response to more orthodox therapy is much better than that of adults.

Prednisone doses up to 3 gm. daily were used by Hill, who reported an overall complete remission rate of 75 per cent in 16 cases of acute leukaemia (Hill, 1957), and although toxic effects were again troublesome, he regarded massive dosage as justified when a rapid response was urgently required and in resistant or terminal cases after other modes of therapy had failed.

Blood or plasma transfusion

Blood transfusion is commonly regarded as a supportive ancillary measure in the management of leukaemia, but in acute leukaemia there is now substantial evidence that

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CHAPTER 11

GENERAL PRINCIPLES OF THERAPY

III. Symptomatic and Supportive Treatment

DESPITE the advances in radiotherapy and selective chemotherapy, leukaemia re an ultimately fatal disease, the course of which, however palliated, is beset from ti time with serious symptomatic problems requiring *ad hoc* treatment. In this cl consideration will be given to some of the more important of these problems, includi emotional background in both patients and relatives, the pathogenesis and treatm haemorrhage, anaemia and infections, and the management of surgical emergencie pregnancies.

Emotional problems

Once an unequivocal diagnosis of leukaemia has been made, the position shou described truthfully to the patient's relatives. Prognosis must necessarily be guar view of the spontaneous variations in the severity and course of the disease and the p predictable results of therapy, but emphasis may reasonably be given to the unrem research for more effective treatment at present being actively pursued in many c and the possibility that further significant advances may be made at any time. I way something can be done to temper the emotional shock experienced by the pat family. Assurance must of course be given that everything possible, with ava methods, will be done to preserve the patient's comfort and freedom from distr symptoms.

When the patient is an adult some direct explanation of his disease is required. much he should be told, and at what stage, will depend on his character, personalit convictions. He should certainly be told the truth about his condition, but not neces the whole truth, at least initially. The explanation may be given that he has deve a blood disease for which there is as yet no permanent cure, but which is often ame to treatment for long periods under careful medical supervision. Direct questions ar answered directly, though with as optimistic a bias as is compatible with honest particular the fatal nature of leukaemia, in the existing state of medical knowledge, s not be denied; it is a patient's right to know when he is suffering from a fatal dise

Emotional disturbances may be expected to occur in patients of any age who ar to appreciate the gravity of their illness, whether this has been frankly discussed wi physician or not. There is evidence, however, that certain psychological abnormalit leukaemic adults may have existed before the appearance of the somatic disease an perhaps have predisposed to its development. Greene (1954) and Greene, Youn Swisher (1956) reported studies on 20 males and 32 females with leukaemia or mali lymphoma, from which they concluded that the majority of patients had suffered

various types of personal loss or separation from a key object or goal, with resulting depression, sadness or hopelessness, and sometimes fatigue, weakness, anorexia and nausea for weeks or months before apparent onset of the somatic disease. Depressive responses, hysterical and anxiety reactions and vegetative neuroses occurring during the established disease may therefore be partially an extension of pre-existing psychological disorder, exacerbated by an awareness of serious somatic illness. While symptoms of this kind are often present in a mild form, they rarely present a major problem, and indeed the courage, good humour and endurance of most adult patients with leukaemia is most impressive.

With leukaemic children the problems are of a different kind. In studies of the psychological aspects of management of children with leukaemia and other malignant diseases, Richmond and Waisman (1955) and Bernard and Alby (1956) noted that, while some patients manifested anxiety, and reactions varied of course with age and level of understanding, most children were ignorant of the idea of personal death and showed passive acceptance of their disease and resignation to it. Some even appeared slightly melancholic. The psychological reactions of the parents were far more dramatic, with the display of strong emotions of fear, lack of acceptance, and culpability. The importance and nature of these reactions and the part the physician can play in their prevention or amelioration have been discussed extensively by Bozeman, Orbach and Sutherland (1955). They conducted a study of the adaptation of 20 mothers to the threatened loss of their children, aged 1½ to 6½ years, from acute leukaemia. They found that the diagnosis of leukaemia had a devastating impact on all mothers, manifested by hostility towards doctors, failure to accept the implications of the diagnosis, frantic attempts to disprove its accuracy by seeking other medical opinions, and inability to comprehend explanatory information. Many mothers expressed guilt and believed themselves personally responsible for the onset of disease by reason of real or imagined failures in devotion to and care and protection of the child. Acute separation anxiety was present in every case during the initial admission to hospital, and the mother's fears increased those of the child. Emotional support from family, neighbours and friends was an urgent requirement, but was rarely adequately supplied. Maternal grandmothers in particular, with few exceptions, failed to provide sympathy and support and appeared reluctant to give practical help with household problems.

The physician's responsibility cannot therefore be confined to the treatment of the child, but must include efforts to relieve the parents' reactions by establishing a sympathetic and supportive relationship with them. They should not be told of the suspected diagnosis until it has been verified with certainty, and the doctor must expect to encounter resentment and hostility and be prepared to meet this without offence or indifference. Many parents will seek to refute the diagnosis by alternative consultations and the physician should not be antagonistic to this natural desire. Feelings of guilt should be alleviated by convincing reassurance, and questions concerning the nature and genesis of the disease should be answered fully and patiently.

Bozeman and her associates noted a strong desire on the part of all parents to prolong the lives of their children as much as possible, even when they were aware of the inevitable outcome. They grasped eagerly at the possibility of experimental or research therapy. Active treatment helped to sustain hope and, in the terminal stages of the illness, enabled

the parents to feel that they had not neglected any step which might have been beneficial. Fortunately the treatment of leukaemia can be conducted largely on an out-patient basis, and this reduces the separation fears, but when admission to hospital is imperative the parents should have ready access to the child, and the mother should be enabled, if possible, to continue maternal activities such as feeding, comforting and playing with the child with the full co-operation of the hospital staff.

An intelligent and sympathetic appreciation of the emotional disturbances induced in patients with leukaemia and in their relatives can do no more than minimize reactions which are inevitably severe, but whatever measures may alleviate mental suffering and distress, abolish feelings of guilt and reduce the anxiety of separation must be regarded as an essential part of the physician's duty in his general management of the disease.

Emotional difficulties are most prominent, of course, in acute leukaemia, while in chronic forms of the disease the more insidious course gives better opportunity for gradual adaptation both by the patient and by the relatives. Moreover, since chronic leukaemias are rare in childhood, acute parental anxiety is less likely to occur. Nevertheless, the need for constant encouragement and sympathy remains very important, particularly at times of relapse, and the patient and his family must always be made to feel that everything possible is being done for him, that his progress is under constant and positive medical control, and that the hope of inducing further remissions and prolonging life will not be abandoned.

The pathogenesis and control of haemorrhage

A bleeding tendency, ranging in severity from an increased liability to bruise after minor trauma to the spontaneous development of widespread petechiae and ecchymoses accompanied by haemorrhages from mucous membranes, is frequently observed in acute leukaemia and is not uncommon at some stages of chronic leukaemia. Thrombocytopenia is almost invariably present when bleeding occurs, but there is evidence that other factors may play an important part in the pathogenesis of bleeding.

Freeman and Hyde (1952) studied the platelet levels, prothrombin activity and heparin-protamine titres in bleeding and non-bleeding leukaemic children and in a group of normal children. Clinical evidence of bleeding was not found in patients with platelet counts above 55,000 per cu. mm., whereas most of those with lower counts did show spontaneous petechiae and ecchymoses or frank haemorrhages. Among 351 blood specimens examined, 94 from non-bleeding individuals showed platelet counts above 55,000 per cu. mm., and 221 from patients with clinical signs of bleeding had platelet counts below this level. In the remaining 36 instances, however, there was no evidence of bleeding although the platelet counts were below 55,000 per cu. mm. While platelet deficiency therefore appeared to be essential if bleeding were to occur, examples of critically low platelet counts in patients who nevertheless did not bleed were not uncommon. Prolongation of one-stage prothrombin times was found in about half of the patients with bleeding, but this coagulation defect was not further analysed. Heparin-protamine titrations to measure the level of circulating anticoagulants with heparin-like activity were performed, but although raised titres were found in 127 of 239 blood specimens from leukaemic patients, bleeding occurred as often among those with normal levels as among those with

elevated titres. Moreover, identical means and similar distributions of heparin-protamine titres were found in both bleeding and non-bleeding groups.

The possibility that infection might influence the development of haemorrhage in thrombocytopenic leukaemic patients was suggested by the observation that fever was more common during periods of bleeding than at other times (Freeman and Buckley, 1954). Secondary infection was usually present, and since it has long been known that bacterial extracts or autolysates may sometimes cause thrombocytopenic bleeding (Julianelle and Reimann, 1926) and may inhibit coagulation *in vitro* (Goodner, 1933), it seemed possible that the bacterial invaders might contribute to the failure of haemostasis. Freeman (1952) showed that bacterial polysaccharides from cultures of Friedländers bacillus and *Serratia marcescens* had an anticoagulant effect on normal plasma from leukaemic patients. Freeman and Buckley (1954) measured the protein-bound serum polysaccharide in 57 leukaemic children with platelet counts below 60,000 per cu. mm. and showed that the risk of bleeding increased directly with the polysaccharide level at low platelet concentrations, although the correlation fell off when platelets were above 25,000 per cu. mm. Platelets and polysaccharides varied independently, but both appeared important in the haemorrhagic diathesis of leukaemia. The authors regarded the increase in polysaccharides as a result of secondary infection with fever rather than as a manifestation of leukaemic cell proliferation, since individuals with advanced leukaemia, but without infection or bleeding, had normal polysaccharide levels.

Fibrinolysis and hypofibrinogenaemia have been observed occasionally in bleeding leukaemic patients, but neither of these abnormalities is common (Janbon, Bertrand and Mironze, 1948; Giraud *et al.*, 1954; Cooperberg and Neiman, 1955; Pisciotta and Schultz, 1955). In most cases the patients have also been severely thrombocytopenic.

The multiplicity of factors occasionally incriminated in the genesis of leukaemic bleeding and the lack of any single consistent and satisfying explanation for this frequent and serious complication led Lewis and her associates (1957) to conduct a battery of tests of haemostatic function in 39 patients with various forms of leukaemia and to compare the results with those of similar tests in 24 patients with thrombocytopenia arising from different causes. The tests included platelet counts, examination of bone-marrow aspirates for megakaryocytes, measurement of clot retraction, prothrombin consumption, and platelet thromboplastic and accelerator activity, serotonin assays, tourniquet tests and tests of bleeding time. Plasma studies included determination of clotting times, 1-stage prothrombin times, plasma thrombin times, prothrombin, factor V and factor VII content, and assays of plasma thromboplastin component (Christmas factor) and fibrinogen. Search was also made for fibrinolysins and platelet agglutinins. "Even after this rather extensive and painstaking study", the authors wryly observed, "no simple answer is apparent for the question: Why do leukaemic patients bleed?" Depression of the platelet count and the level of plasma factor V were frequently noted in the leukaemic group, but neither of these abnormalities correlated well with the degree of bleeding. Megakaryocytes were usually few in leukaemia when the platelet count was low, in contrast to the general finding of megakaryocytic hyperplasia in idiopathic thrombocytopenic purpura. Other differences between these two groups were the results of tourniquet tests, usually normal in leukaemia and strongly positive in I.T.P., and of bleeding times, which were less often prolonged in leukaemia. Serotonin levels were low in leukaemic patients, but not in those

with I.T.P. Evidence of fibrinolysis was found in 5 of the 39 leukaemic patients, but in 3 of these the bleeding was only slight or moderate.

The majority of investigations carried out on the haemostatic mechanism in leukaemia have been done in patients under treatment with steroids, antimetabolites, cytotoxic drugs or X-rays, and it has not been possible to exclude or evaluate with accuracy the effects of such therapy on platelet activity and plasma factors. Among the patients studied by Lewis and her colleagues a very striking correlation was seen between the general clinical status and the tendency to bleed. Clinical deterioration in both acute and chronic leukaemias was invariably found when bleeding occurred, and patients in good remission did not bleed. This observation, which is in conformity with the impressions of most clinicians concerned with leukaemic patients, underlines the importance of regarding bleeding in leukaemia as an integral part of the disease process. The best treatment for this symptom is therefore to induce remission of leukaemic proliferation, and one need not hesitate to use potentially thrombocytopenogenic drugs in the presence of leukaemic, thrombocytopenic bleeding, if there is a reasonable expectation of inducing remission by their action. When thrombocytopenia and haemorrhages develop during treatment with cytotoxic agents it is not always easy to decide whether the platelet fall is the result of drug action or of leukaemic relapse. If the drug is responsible, there will usually be evidence of pancytopenia and marrow hypoplasia, and treatment should be stopped or changed, but if intensive leukaemic activity is present, continuation of cytotoxic therapy may still be effective against both the leukaemic relapse and the accompanying bleeding.

When remission cannot be induced the bleeding can only rarely be controlled. Temporary improvement may follow transfusion with fresh blood taken into plastic bags or siliconized apparatus to preserve platelets, and treatment with prednisone may also be helpful in improving capillary resistance although the platelet level does not usually increase unless some degree of remission in the leukaemic process is induced. Direct local haemostatic measures, such as cauterization of bleeding points on the nasal septum, or the application of gauze packing or pressure bandages, are generally fruitless unless accompanied by successful anti-leukaemic therapy. The chief value of all these procedures lies in their contribution to temporary control of serious haemorrhage during the early stages of treatment with effective chemotherapy.

The pathogenesis and control of anaemia

Anaemia is almost invariably present at some stage of the disease in all forms of leukaemia, but the way in which it is produced is not yet clear. When a haemorrhagic state exists, blood loss may contribute to the genesis of anaemia, and this factor is commonly important in acute leukaemia, but in most patients with chronic leukaemia and in many with acute, anaemia develops in the absence of overt haemorrhage. Deficiency of accepted haematinic factors such as iron, folic acid, vitamin B_{12}, and ascorbic acid, appears pathogenetically unimportant, since there is no response to their administration, and the same is true for hormones such as thyroxine.

Mechanical overcrowding of the marrow with leucopoietic tissue was long believed to reduce erythropoietic activity and so to produce a hypoplastic or aplastic type of anaemia, and the great increase in the myeloid-erythroid ratio in marrow aspirates and sections lent support to this hypothesis. The rise in haemoglobin level and red cell count commonly

found when white cell proliferation is reduced by therapy is also in accord with the idea of overcrowding. Nevertheless, the early observations of Jaffé (1935) and of Kress (1934) drew attention to the frequent presence of phenomena suggesting increased destruction of red cells, and the existence of active erythropoiesis. Haemosiderosis, erythrocyte phagocytosis and high excretion of bilirubin were described. These findings were not generally confirmed, however, and most investigators before 1949 felt that frank haemolysis was only rarely a contributory factor in the anaemia of leukaemia (Collins and Rose, 1948).

Fresh light was thrown on the problem by the studies of Huff and his associates (1949, 1950), who used radioactive iron to investigate plasma and red cell iron turnover in various blood diseases and found that the rate of red cell production in most cases of chronic leukaemia was, surprisingly, not diminished, but normal or even increased. This finding, in association with anaemia and low total erythrocyte mass, suggested that anaemic, leukaemic patients must have a shortened red cell life-span. Experimental support for this suggestion was obtained by direct measurement of the red cell life in leukaemic patients, using C^{14}-labelled glycine (Berlin, Lawrence and Lee, 1954). In five cases of chronic granulocytic leukaemia a finite but shortened life-span of 70 to 100 days was found, in keeping with the existence of an intrinsic defect in the red cells. In two patients with chronic lymphocytic leukaemia finite life-spans of 102 and 113 days, little below normal, were found, but in a third patient random destruction of red cells took place, with average life-span as low as 18 days, suggesting the possible existence of an extracorpuscular haemolytic factor. Since 1950 several other workers have confirmed the shortening of erythrocyte life and the normal or increased rate of erythropoiesis in patients with chronic leukaemias and anaemia (Ross, 1951; Berlin, R., 1951; Brown, Elliott and Young, 1951; Ross *et al.*, 1954). The existence of auto-immune haemolytic anaemia in a considerable proportion of patients with chronic lymphocytic leukaemia has been discussed previously (see Chapter 7), and it is in such patients that very short red cell life-spans, with random destruction, may be expected. When an auto-immune state is not present in chronic lymphocytic leukaemia, anaemia is less striking and the erythrocyte life-span is generally finite and nearly normal; in these cases anaemia may in fact be due to encroachment of proliferating lymphocytes and reduction in red cell formation and release. The pathogenesis of anaemia in chronic granulocytic leukaemia is still not clear. An intrinsic red cell defect of uncertain nature may be present, as proposed by N. I. Berlin and his associates in view of their finding a consistently shortened finite red cell life-span, but the observations of R. Berlin (1951), with the Ashby technique of differential agglutination, suggest that transfused normal cells also have a much shortened survival in the circulation of patients with chronic granulocytic leukaemia and this would indicate an extrinsic destructive mechanism, perhaps related to the degree of splenomegaly.

In acute leukaemia, anaemia appears again most often to be due to a shortening of red cell survival, with normal or accelerated erythropoiesis (Schapira *et al.*, 1954; Boiron *et al.*, 1955). Both the patient's own cells and transfused normal cells have been found to be destroyed prematurely, and an extrinsic destructive mechanism must presumably be responsible. Evidence of auto-immune activity is rare in acute leukaemia, although isolated instances are recorded (Frumin and Kohn, 1955), but an abnormal haemolytic system of possible importance has been observed in the plasma of some patients with

acute leukaemia by Crosby and Benjamin (1957). This haemolytic system can be detected by incubation of sterile blood and differs from the mechanisms operative in spherocytosis, complement-antibody haemolysis and paroxysmal nocturnal haemoglobinuria. It occurs not only in acute leukaemia, but also in some patients with lymphomata or other disseminated neoplastic states. The significance of this system in the pathogenesis of anaemia has not yet been established.

In all forms of leukaemia, but especially in the acute disease, anaemia may of course be due to marrow aplasia, when a general aplastic state arises during the course of the illness, and it is then accompanied by leucopenia and thrombocytopenia, and marrow aspirates are poorly cellular. A similar picture may be produced by aggressive therapy, and the development of an aplastic phase with associated anaemia often precedes the onset of remission and return to normal haemopoietic activity.

With regard to treatment of anaemia in leukaemia, the most effective measures, as with leukaemic thrombocytopenia, are those directed against the leukaemic process. Control of leukaemic proliferation by chemotherapy or radiotherapy is nearly always paralleled by a rise of haemoglobin to normal values, and, in general, no other effective treatment for the characteristic anaemia exists. Blood transfusion may be of temporary benefit and sometimes enables a patient to survive long enough for more specific anti-leukaemic therapy to take effect, but it is rarely possible to maintain a sustained high level of haemoglobin for any worthwhile period by transfusion alone, unless the transfusion itself induces remission, as it may occasionally do in acute leukaemia. When an auto-immune haemolytic process is present, treatment with prednisone in doses from 5 to 30 mgm. daily is usually successful in reducing or abolishing the contribution of this process to the genesis of anaemia and may therefore be highly effective in anaemic patients with chronic lymphocytic leukaemia. In acute leukaemias prednisone improves anaemia only when general remission is induced, and in chronic granulocytic leukaemia it is usually ineffective.

The value of splenectomy in the control of anaemia in leukaemic patients has been a subject of dispute for many years. Immediate operative mortality was high in the earlier cases treated by splenectomy; Hagen, in 1900, collected reports of 42 patients with leukaemia who had been subjected to splenectomy and found that 38 of them died as a result of the operation. Advances in surgical technique and the use of pre-operative radiation to reduce the size of the spleen brought the operative mortality below 5 per cent, and with suitable selection of cases the figure would now be very much lower. While the operation is unlikely to be immediately harmful, its possible beneficial effects have not been clearly established. R. Berlin (1951) carefully reviewed the literature on splenectomy in leukaemia and presented the results of red cell survival studies before and after operation in a series of patients with chronic leukaemia. He found some correlation between the initial reduction in erythrocyte life-span and the extent of splenomegaly, and a return towards normal erythrocyte survival after operation. In his view splenectomy was unquestionably of value in those cases where considerable splenic enlargement was present in association with a normochromic anaemia, moderate reticulocytosis, persistent urobilinuria, increased faecal stercobilin, and curtailment of the red cell survival time. These circumstances were most likely to arise in chronic granulocytic leukaemia, and he recommended that the operation should be performed as early as possible in the course of the disease and should be preceded by the induction of as full a remission as could be

obtained by radiation or chemotherapy. Splenectomy might then be expected to improve anaemia and perhaps enable longer remissions and a greater average length of life from onset of the disease to be achieved. The available reports do not, however, provide consistent evidence that any significant prolongation of life results from splenectomy as compared with modern methods of chemotherapeutic control, since no substantial series of cases with adequate follow-up has been studied in recent years. Most physicians undoubtedly believe that splenectomy has no place in the routine management of chronic granulocytic leukaemia, nor, indeed, of any other form of leukaemia, but should be reserved for consideration in patients with intractable anaemia and splenomegaly who have failed to respond to medical measures. In such patients dramatic relief may occasionally be obtained (Fisher, Welch and Dameshek, 1952).

Fever and infection in leukaemia

Fever is an extremely common feature of acute leukaemia and frequently develops at some stage in the chronic leukaemias. While pyrexia has often been regarded as an integral part of the disease, related to increased metabolic activity, several recent studies have thrown doubt on this conception, suggesting instead that fever in leukaemia is almost invariably secondary to infection. Louis, Limarzi and Lepper (1956) reviewed the clinical course of 41 patients with leukaemia (32 acute and 9 chronic) who exhibited a total of 82 febrile episodes. Bacterial cultures were made in 42 episodes and proved positive in 38, and in a considerable proportion of febrile episodes which were not studied bacteriologically improvement took place with or without antibiotic therapy. The most frequent sites of infection were the blood, the respiratory tract and the ear, and the most common organisms *Streptococcus viridans*, *Pseudomonas* and coliforms. The onset of infections could not be correlated with the total leucocyte count, the degree of remission or the type of therapy. The authors concluded that infection was the primary cause of fever in leukaemia.

Silver and his colleagues (1958) carried out a more detailed and comprehensive study, with bacterial, viral and fungal techniques, in 36 consecutive patients with acute leukaemia. Only 2 of the patients remained free from fever throughout the period of study, and 92 febrile episodes occurred among the remainder. Infection was established as the cause of 44 of these episodes, the most common illnesses being pharnygitis, pyelonephritis and septicaemia, and the most common organisms *Escherichia coli*, coagulase-positive staphylococci and *Pseudomonas aeruginosa*. With appropriate antibiotic treatment, 34 of these episodes cleared up satisfactorily, but in 10 the infection could not be controlled and terminated fatally. In a further 15 febrile episodes, although bacterial or viral infection was not proved, there was a presumptive relationship to respiratory tract infection, with clinical and radiographic evidence of disease, and in every case the fever subsided during antibiotic therapy. An infective cause could not be found for the remaining 33 febrile attacks, but nevertheless 19 of them responded to penicillin, streptomycin or tetracycline, and 8 of the 14 which did not respond eventually terminated in serious overt bacterial infection.

In analysing and discussing their data, Silver and his associates noted that fever related to demonstrable infection was relatively more common than fever of undetermined origin in adults, whereas the reverse was true in children. Infective causes for fever were

:iently common in both groups, however, to justify regarding any febrile episode as to infection until the absence of localizing physical signs and negative bacteriological radiological examinations proved otherwise. The response of bacterial infections to biotics was dictated not only by the sensitivity of the organisms but also by the natological state; infections could be more easily controlled when the leukaemic ess showed a degree of remission, and were most refractory in severe leukaemic ose.

ne high incidence of infections, often with organisms of relatively low virulence, in aemia, and the obvious disturbance of the haemopoietic and reticulo-endothelial ems in that disease have long suggested an impairment of the cellular and humoral hanisms of anti-bacterial defence. The cellular response to local infections was studied affé (1932), who found that leukaemic patients with mature granulocytes in the circuon reacted with a morphologically normal inflammatory exudate, but when granulosis was exhausted there was no leucocytic defence reaction. Immature granulocytes not appear in inflamed areas and played no apparent part in local defence. Subsequent stigators have confirmed these observations (Silver *et al.*, 1958), although there has a some dispute over the phagocytic capacity of mature neutrophils in leukaemia. shberg (1939) noted that infection might develop in acute leukaemia, when the mature trophil count was high, and found some evidence of impaired phagocytic activity of se neutrophils. It is certainly true that severe infections may develop in patients with a absolute neutrophil counts, although such patients usually have very much higher nbers of primitive cells, and, on the other hand, patients with very low neutrophil nts sometimes remain relatively free from infection. Neutrophils in leukaemia might refore be thought to have little functional importance in resisting invasion by pyogenic anisms. However, the experiments of Brande, Feltes and Brooks (1954) and of Silver l his associates (1957) have demonstrated unimpaired phagocytosis of various organisms luding staphylococci and *Brucella*, by mature neutrophils from the blood of patients h leukaemia, despite the lack of a clear correlation between the level of mature neutrols and liability to infection.

Measurements of antibody response to injected antigens have shown a normal capacity react in chronic granulocytic leukaemia, but a poor response in chronic lymphocytic kaemia (Larson and Tomlinson, 1953), and this finding may partly explain the greater bility to infections observed in the lymphocytic as compared with the granulocytic m of chronic leukaemia (Wintrobe and Hasenbush, 1939). In the acute disease, the tibody reactions of a group of patients studied by Silver and his co-workers (1958) were, average, less than those of a control group of normal subjects, but some leukaemic tients reacted strongly to injected antigens and the extent of response in any individual uld not be correlated with the observed frequency or severity of infections. This riability in antibody response is paralleled by a similar variability in levels of serum mma-globulin, but it is interesting to note, in connection with the susceptibility of tients with chronic lymphocytic leukaemia to infections, that several investigators have ınd striking decreases in the serum gamma-globulin levels in a high proportion (40 to per cent) of patients with this form of leukaemia (Creyssel *et al.*, 1957; Jim, 1957; ıdson and Wilson, 1957). There was some evidence that infections occurred more :quently when hypogammaglobulinaemia was present, but both features were more

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marked in the later stages of the disease. In chronic granulocytic leukaemia and in acute leukaemias, marked changes in serum globulin levels were uncommon.

An additional factor which may play a part in determining predisposition to infection in leukaemia is properdin. The techniques at present used for properdin assay are rather elaborate and there has been a certain lack of uniformity in results reported from different laboratories. Hinz (1957) found normal levels of serum properdin in patients with leukaemia, and Rottino, Levy and Conte (1958) found little depression in the few leukaemic sera they assayed. On the other hand, Eyquem and Tullis (1957), from a study of 28 patients, reported low values of properdin in chronic lymphocytic leukaemia but normal values in chronic granulocytic leukaemia, while Hunter and Hill (1958) found very low levels in 8 sera from acute lymphoblastic leukaemia and moderately low values in 7 patients with acute myeloblastic and 3 with chronic lymphocytic leukaemia. The patients in most of these studies included treated and untreated persons and those in various phases of disease, and no correlation has emerged between properdin levels and severity of the disease process. Nevertheless, it does appear that properdin values are lowest in those types of leukaemia, the acute and chronic lymphocytic forms, in which infection is most prevalent, and Hunter and Hill suggest that the appropriate administration of properdin may eventually prove vital in treating leukaemia.

The management of infections in leukaemia should follow accepted general principles. When a causative organism has been identified, local and systemic treatment with antibiotics or other suitable agents chosen according to the sensitivity of the organism is frequently successful. Persistent fever of undetermined origin may respond to antibiotics, and their administration for 7 to 10 days is worth a trial. If possible, intramuscular injections should be avoided in leukaemic patients with a severe bleeding tendency, since tissue haemorrhages may occur at the site of injection, and orally administered antibiotics are therefore to be preferred. The use of prophylactic antibiotics during periods of relapse, in an attempt to prevent the onset of infection, is of rather doubtful value. Silver and his associates (1958) observed that many of the infections encountered in their study were due to penicillin-resistant staphylococci, or to *Pseudomonas*, and would probably not have been prevented by the usual dose and type of antibiotics used in prophylaxis. Long-term prophylactic use of antibiotics is certainly inadvisable, because of the risks of superinfection with fungi following upon alterations in the normal bacterial flora of the alimentary tract.

The incidence of secondary fungus infections in leukaemia has increased conspicuously in recent years. Keye and Magee (1956) surveyed retrospectively the incidence of fungus infections complicating other diseases among 15,845 patients over the period 1919 to 1955. A high proportion of these infections since 1947 occurred in leukaemic patients, but no instances of mycotic infection in patients with leukaemia or malignant lymphoma were found in this series before 1947. In the last two years of the survey (1954 and 1955) complicating fungus infections were found in no less than 20 per cent of patients with leukaemia and lymphoma. Histoplasmosis, crytococcosis, mucormycosis, candidiasis and aspergillosis were all involved in the increase, candidiasis being the most common superinfection. Similar findings were reported by Stefanini and Allegra (1957), who observed fungus infections in only 3 per cent of acute leukaemia patients during the period 1943 to 1947, but 14 per cent and 22 per cent respectively in the periods 1947 to 1949 and 1954

to 1956. While some part of the increase may be due to improved diagnosis and to longer survival of leukaemic patients with a consequently greater opportunity for the development of complications, there seems little doubt that modern forms of treatment heighten susceptibility to fungus infections. Antibiotics, especially the tetracyclines, certainly encourage the emergence of fungi, either by directly enhancing growth or by destroying intestinal bacteria which normally exert a controlling influence on the fungal flora of the gut (Seligmann, 1953; Huppert, MacPherson and Cazin, 1953; Oeding and Austerheim, 1954). Adrenocorticotrophic hormone, cortisone and related steroids also appear to decrease resistance to the spread of fungus infections (Mankowski and Littleton, 1954). Finally, antimetabolic and cytotoxic drugs used in the chemotherapy of leukaemia commonly produce leucopenia which may in turn increase susceptibility to mycoses. Since agents in all these classes were introduced for the treatment of leukaemia in rapid succession after 1948, it is not easy to ascribe the precipitate increase in fungus infections to the use of any one of them. Probably they all play a part in determining predisposition. Thus, fungi were isolated from 18 of the 36 patients with acute leukaemia studied by Silver *et al.* (1958), and in only half of those showing clinical evidence of fungus disease was there any temporal relationship to antibiotic therapy. Despite the incrimination of therapeutic measures in the pathogenesis of fungus infections, such infections commonly resolve satisfactorily if remission is induced. Moreover, they are usually confined to the pharynx, and even if disseminated cannot be regarded as contraindicating appropriate chemotherapy if there is any hope of remission being achieved. The discomfort due to pharyngeal moniliasis and the risk of local and generalized spread are considerable, however, and active fungicidal therapy should be instituted as soon as the infection has been recognized. A generally effective agent is fortunately available in nystatin (mycostatin, fungicidin), an antibiotic derived from *Streptomyces nousei* (Hazen and Brown, 1950). Nystatin has been shown to be active *in vitro* against many fungi, including the common species of *Candida*, and it is highly effective against experimental infections with *Candida albicans* in the mouse (Brown, Hazen and Mason, 1953). Favourable clinical results in fungus infections of man have been reported, some in cases of superinfection in leukaemia. Bernard, Mathé and Drouhet (1955) recorded good results in 6 cases, 3 with acute leukaemia, 2 with lymphocytic, and 1 with granulocytic leukaemia. All these patinets had buccal and pharyngeal thrush. Numerous *Candida* organisms were present in the stools of 4 patients and in the urine of 3, while 1 patient had high fever and a positive blood culture of *Candida*. Treatment with nystatin, locally applied to the mouth and pharynx, or given systemically in oral doses of 0·2 to 1·0 gm. daily, equivalent to 500,000 to 2,500,000 units, led to rapid resolution of the mycotic lesions in from 1 to 10 days. Other similar experiences have been reported, and although nystatin is not always effective in fungus infections it is invariably worth trial. It may be conveniently applied as a local paint to lesions in the mouth, but if candidiasis is more widespread, daily oral administration of 3 to 8 nystatin tablets, each containing 500,000 units, is advisable.

That appropriate treatment of infections with antibiotics successfully prolongs the life of many leukaemic patients is unquestionable. Bierman and associates (1950) found that the free use of blood transfusions and antibiotics had increased the average survival time of children with acute leukaemia from 5·6 to 8·9 months, before the introduction of specific anti-leukaemic chemotherapy. Southam and colleagues (1951) similarly observed

a steady increase in survival times over the years 1935 to 1948, in parallel with the increasing use of supportive measures such as blood transfusion and systemic anti-bacterial chemotherapy with sulphonamides, penicillin and other antibiotics. While antibiotics therefore improve survival, there is no evidence that they play any significant part in inducing remissions of the leukaemic process, and their use must be regarded as purely supportive in the overall management of the disease.

Perhaps the most puzzling and fascinating aspect of the relationship between acute infections and leukaemia is the occasional development of clinical and haematological remission as an apparent consequence of infection. Isolated reports of this phenomenon have appeared in considerable numbers, and as early as 1904 Dock was able to collect sixty-five references to remissions in leukaemia following acute infections. It is, of course, true, as Wintrobe and Hasenbush (1939) pointed out, that the great majority of infections do not induce remission and that remissions following infection rarely occur in chronic leukaemia, but a surprisingly high proportion of remissions in acute leukaemia in the years before effective chemotherapy seem to have had a temporal relation to a previous severe infective episode. Diamond and Luhby (1951) reviewed the case histories of 270 children with acute leukaemia and found among them 26 examples of remission, preceded in each case by leukopenia and marrow hypoplasia. Severe infections were found to have occurred immediately before the hypoplastic phase in 11 of 12 "completely" remitting patients and in 10 of the 14 showing partial remissions. The question was further studied by Southam and associates (1951), who noted that remissions in patients with acute leukaemia treated with supportive therapy alone were frequently, but not always, preceded by a period of fever. These workers found it difficult to accept any causal relation between infection and remission, but nevertheless speculated that nutritional deficiency during a period of debilitating fever might deplete metabolic reserves and so lead to remission, or, alternatively, that the adrenal stress of a febrile illness might stimulate the liberation of adrenal cortical hormones active against the leukaemic process. Bierman and his colleagues (1953) reported marked clinical and haematological remissions of acute lymphoblastic leukaemia in 2 children with staphylococcal septicaemia and in 1 child with varicella; injection of feline panleukopenia virus intramuscularly into a further 6 children with acute leukaemia produced febrile episodes, followed in 1 child by a good remission and in another by a partial improvement. Experimental attempts to set up infection which might induce remission have also been carried out by Taylor (1953), who observed a clinical remission in an adult patient with acute monocytic leukaemia coincident with the appearance of glandular-fever type cells in the peripheral blood and a positive Paul-Bunnell heterophil antibody reaction in the serum. Injections of 2 to 10 ml. of glandular-fever serum were therefore given to 5 further patients with monocytic leukaemia. In 2 cases no evidence of infection appeared and the leukaemia ran an unmodified course, but in the other 3 patients positive Paul-Bunnell reactions developed and glandular-fever cells appeared in the peripheral blood, while at the same time the leucocyte counts fell, with a reduction in the percentage of monoblasts, and there was a tendency for haemoglobin levels and platelet counts to rise.

The mechanism of post-infective remissions and the possible therapeutic value of induced infections remain speculative, and the scope for experimental studies in man is clearly restricted, at least to patients in the terminal stages, because of the potential

danger that artificial infection may prove lethal and since chemotherapeutic means of treatment are so often effective in the earlier stages of the disease. Useful information is, however, being derived from animal experiments, such as those of Nadel and Haas (1956) on the retarding effects of lymphocytic choriomeningitis virus on the course of transplantable leukaemia in guinea-pigs, and an explanation and even perhaps a therapeutic use for this curious relation between infection and remission in leukaemia may eventually be found.

The management of surgical emergencies

Leukaemic patients in remission or under satisfactory therapeutic control tolerate surgery reasonably well, and elective operations such as splenectomy, prostatectomy (Swersie, 1956), and even lung resection (Effenberger and Schulte, 1957) may be performed without undue risk. Acute emergencies calling for immediate surgery can be dealt with in an orthodox way without serious risk of disaster in times of remission, but in patients having florid leukaemia, especially acute forms, the risks of post-operative infection and haemorrhage are great. Transfusions of fresh blood and the provision of prophylactic antibiotic cover at the time of operation and during the next week or ten days help to minimize these dangers, but even minor surgery should not be lightly undertaken in leukaemic patients in relapse.

Leukaemia and pregnancy

Women with leukaemia rarely become pregnant, and since leukaemia is not a common disease its onset during pregnancy is correspondingly rare. Nevertheless, examples of the association of leukaemia and pregnancy have been sporadically reported for many years and detailed information on many aspects of the subject can be derived from successive reviews of the literature (Grier and Richter, 1939; McGoldrick and Lapp, 1943; Erf, 1947; Li, McBride and Mettier, 1947; Williams, 1948; Allan, 1954; Gillim, 1955; Sheehy, 1958; Yahia, Human and Phillips, 1958). From the more recent of these reviews it is clear that well over 150 case reports of pregnancy with leukaemia have now been published. Of the patients described, about 56 per cent had chronic granulocytic leukaemia, 41 per cent acute leukaemia, and only about 3 per cent chronic lymphocytic leukaemia. This distribution is to be expected from the known frequency and duration of the different forms of leukaemia in women during the child-bearing period of life. In 89 patients with chronic granulocytic leukaemia and pregnancy, reviewed by Sheehy (1958), the diagnosis of leukaemia had been established before the onset of pregnancy in 66 and during the pregnancy in 23, but the disease may well have been present undiagnosed for some months in many patients in the latter group. In patients with acute leukaemia the reverse of this picture is true; the great majority develop the disease during established pregnancy, and it is very rare for a patient with known acute leukaemia to become pregnant.

As far as the effect of pregnancy on the leukaemic patient is concerned, there is no convincing evidence that the course of the disease is influenced in any way. Acute leukaemia in the adult tends to run so rapid a course that it is not surprising to find nearly all gravid women with this form of the disease dying before or very soon after delivery. Their average survival from onset of the leukaemia is not very different from that of non-pregnant adults with the same disease, and pregnancy does not therefore appear to exert

any deleterious effect. The physical strain of parturition and the risk of severe haemorrhage at this time have sometimes led to early death after delivery, but other patients with acute leukaemia have survived this experience surprisingly well, only to die of their disease within a few weeks or months. In chronic leukaemias again many patients withstand pregnancy and parturition well, and although relapse or myeloblastic transformation has sometimes been noted to follow shortly after delivery, the majority of the collected case reports do not suggest that pregnancy can often be held to have precipitated the terminal development.

The effects of maternal leukaemia on the foetus are not easy to assess from the figures available. Certainly the disease never appears to have been transmitted from mother to child. Examination of the blood of at least 60 viable infants born of leukaemic mothers, and of sections from the tissues of many children born dead, has shown no instance of leukaemic proliferation. One detailed haematological study of the blood and bone marrow of a mother with subacute lymphocytic leukaemia and of her viable infant revealed no persistent maternal influence on the leucocytes of the child (Bierman *et al.*, 1956). Adequate follow-up studies of the children of mothers with leukaemia during pregnancy have not been reported, so that it is not possible to state dogmatically that such children have no increased liability to develop the disease in later life, but, if this were the case, it seems probable that the association of circumstances would have been observed and reported. Although the leukaemic process does not itself directly affect the foetus, the debility, anaemia and tendency to infections in the mother exert a proportional effect on the foetal health, and the more seriously ill the mother the more likely is foetal death. In acute leukaemia previous records suggest that more than 50 per cent of infants are stillborn or die in the neonatal period, whereas in chronic leukaemia about 75 per cent survive. Since the chances of her bearing a healthy infant are high when the mother remains in good remission during gestation and parturition, the foetal mortality figures derived from case records of many years past may be over-pessimistic; with more effective modern methods of control and total care a lower rate of foetal death may reasonably be expected.

The treatment of leukaemia during pregnancy must be designed to produce the fullest possible remission in the mother without harming the foetus. In chronic leukaemia X-rays have sometimes been used cautiously, care being taken to avoid any irradiation of the foetus, and successful delivery of a healthy child has been achieved, but the possibility of indirect radiation effects leading to foetal abnormalities cannot be ignored, and it is perhaps wiser to avoid radiation altogether as a form of therapy during pregnancy. Arsenic, in the form of Fowler's solution, may be used without deleterious effect on the foetus and has been recommended for this situation by several authorities (Angelucci, 1944; Erf, 1947; Whitby, 1956). Supportive transfusions can of course be given as required. Urethane has also been employed, but this drug and the more recent alkylating agents have not been used in enough cases to justify a conclusion as to their merits. Since most antimitotic agents exert a strong action on rapidly dividing embryonic tissues, their use should be avoided whenever possible, and if a decision is taken to use such drugs as busulphan or chlorambucil in chronic leukaemia the dose should be kept to the minimum compatible with moderate remission. The same argument applies to the use of antimetabolites in acute leukaemia. Treatment has been given successfully with 6-mercaptopurine, continued during pregnancy in a dosage of 2·5 mgm. per kg. daily, without pro-

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ducing congenital deformities or haematological abnormalities in the infant (Merskey and Rigal, 1956; Schumacher, 1957), but the risks to the embryo must be considerable even at this dosage, and larger doses would probably have led to abortion or gross deformity. Remissions may be achieved more safely by the use of prednisone or cortisone, and these hormones may enable the pregnancy to be brought satisfactorily to term (Morgan and Reyes, 1956). In general, delivery should be conducted in a normal manner, but if the mother develops a severe and intractable relapse after the seventh month, and the foetus is viable, immediate Caesarean section may save the child (Moloney, Heffernan and Kasdon, 1943; Erf, 1947). Post-mortem surgical delivery has been attempted, but has usually been unsuccessful since the death of the foetus appeared to take place before that of the mother (Grier and Richter, 1939; Almklov and Hatoff, 1946).

The management of pregnancy and leukaemia will clearly vary according to the type and stage of the leukaemia and the time in pregnancy at which it is diagnosed. Specific anti-leukaemic therapy should be administered conservatively with due regard for possible injurious effects on the foetus, but every attempt should be made, by the generous use of supportive and symptomatic treatment directed against anaemia, infection or haemorrhage, to offset disadvantages arising from the inability to use chemotherapy and radiotherapy to their fullest extent. Neither on moral nor on medical grounds is there any case for the deliberate termination of pregnancy in the interests of the mother's health.

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The effects of maternal leukaemia on the foetus are not easy to assess from the figures available. Certainly the disease never appears to have been transmitted from mother to child. Examination of the blood of at least 60 viable infants born of leukaemic mothers, and of sections from the tissues of many children born dead, has shown no instance of leukaemic proliferation. One detailed haematological study of the blood and bone marrow of a mother with subacute lymphocytic leukaemia and of her viable infant revealed no persistent maternal influence on the leucocytes of the child (Bierman *et al.*, 1956). Adequate follow-up studies of the children of mothers with leukaemia during pregnancy have not been reported, so that it is not possible to state dogmatically that such children have no increased liability to develop the disease in later life, but, if this were the case, it seems probable that the association of circumstances would have been observed and reported. Although the leukaemic process does not itself directly affect the foetus, the debility, anaemia and tendency to infections in the mother exert a proportional effect on the foetal health, and the more seriously ill the mother the more likely is foetal death. In acute leukaemia previous records suggest that more than 50 per cent of infants are stillborn or die in the neonatal period, whereas in chronic leukaemia about 75 per cent survive. Since the chances of her bearing a healthy infant are high when the mother remains in good remission during gestation and parturition, the foetal mortality figures derived from case records of many years past may be over-pessimistic; with more effective modern methods of control and total care a lower rate of foetal death may reasonably be expected.

The treatment of leukaemia during pregnancy must be designed to produce the fullest possible remission in the mother without harming the foetus. In chronic leukaemia X-rays have sometimes been used cautiously, care being taken to avoid any irradiation of the foetus, and successful delivery of a healthy child has been achieved, but the possibility of indirect radiation effects leading to foetal abnormalities cannot be ignored, and it is perhaps wiser to avoid radiation altogether as a form of therapy during pregnancy. Arsenic, in the form of Fowler's solution, may be used without deleterious effect on the foetus and has been recommended for this situation by several authorities (Angelucci, 1944; Erf, 1947; Whitby, 1956). Supportive transfusions can of course be given as required. Urethane has also been employed, but this drug and the more recent alkylating agents have not been used in enough cases to justify a conclusion as to their merits. Since most antimitotic agents exert a strong action on rapidly dividing embryonic tissues, their use should be avoided whenever possible, and if a decision is taken to use such drugs as busulphan or chlorambucil in chronic leukaemia the dose should be kept to the minimum compatible with moderate remission. The same argument applies to the use of antimetabolites in acute leukaemia. Treatment has been given successfully with 6-mercaptopurine, continued during pregnancy in a dosage of 2·5 mgm. per kg. daily, without pro-

ducing congenital deformities or haematological abnormalities in the infant (Merskey and Rigal, 1956; Schumacher, 1957), but the risks to the embryo must be considerable even at this dosage, and larger doses would probably have led to abortion or gross deformity. Remissions may be achieved more safely by the use of prednisone or cortisone, and these hormones may enable the pregnancy to be brought satisfactorily to term (Morgan and Reyes, 1956). In general, delivery should be conducted in a normal manner, but if the mother develops a severe and intractable relapse after the seventh month, and the foetus is viable, immediate Caesarean section may save the child (Moloney, Heffernan and Kasdon, 1943; Erf, 1947). Post-mortem surgical delivery has been attempted, but has usually been unsuccessful since the death of the foetus appeared to take place before that of the mother (Grier and Richter, 1939; Almklov and Hatoff, 1946).

The management of pregnancy and leukaemia will clearly vary according to the type and stage of the leukaemia and the time in pregnancy at which it is diagnosed. Specific anti-leukaemic therapy should be administered conservatively with due regard for possible injurious effects on the foetus, but every attempt should be made, by the generous use of supportive and symptomatic treatment directed against anaemia, infection or haemorrhage, to offset disadvantages arising from the inability to use chemotherapy and radiotherapy *to their fullest extent. Neither on moral nor on medical grounds is there any case* for the deliberate termination of pregnancy in the interests of the mother's health.

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CHAPTER 12

ACUTE LEUKAEMIA: CLINICAL ASPECTS

THE three major varieties of acute leukaemia, myeloblastic, lymphoblastic and acute monocytic, have many striking similarities in their clinical manifestations and are often difficult to distinguish one from another. The general clinical discussion of the modes of onset, symptomatology and correlated pathology, differential diagnosis from non-leukaemic disorders, treatment, course and prognosis of these closely related leukaemias will therefore deal with them as a group, while the occasional points of clinical distinction and the more striking morphological differences between members of the group will be emphasized as they arise in the discussion. A series of case reports, illustrating the variety of clinical and haematological features in acute leukaemia, is included at the end of this chapter.

Mode of onset

Acute leukaemias in children and young adults usually have an abrupt onset, with rapid development of multiple symptoms and signs. Pyrexia, lethargy, prostration, headache, and pains in the back and limbs are commonly found as initial symptoms, and lend a resemblance to the onset of a severe infection. Accompanying these general symptoms, however, are rapidly increasing pallor and breathlessness on exertion due to progressive anaemia, and haemorrhages from almost any site, particularly in the form of cutaneous purpura, epistaxes and bleeding from the mucous membranes of the mouth or from the uterus. Another very common and conspicuous feature is the presence of spongy swollen gums, sore throat and necrotic ulceration of the buccal mucosa, and this unpleasant mouth condition is sometimes the symptom which first leads the patient to seek medical advice. Enlargement of lymph glands, liver or spleen may be observed, but is rarely gross and not infrequently absent. Bacterial, viral or fungus infections of the pharynx, respiratory tract or elsewhere are often present from an early stage. Variations in the pattern of initial symptoms depend to some extent on the location and size of leukaemic infiltrates or glandular masses, but even more on the secondary phenomena of bleeding, infection and anaemia. Respiratory obstruction, cough and dyspnoea consequent upon enlargement of the mediastinal glands or thymus may occasionally provide the first symptoms, while in rare cases neurological manifestations secondary to haemorrhage or infiltration in the central nervous system may predominate. Bone and joint involvement may produce a clinical picture like that of acute rheumatic fever. Infection, infiltration or haemorrhage may give rise to conspicuous symptoms in the genito-urinary system, the gastro-intestinal tract, the eyes, ears, nose or larynx, or, indeed, in an almost endless variety of possible sites. It is not, therefore, surprising that a considerable number of patients with acute leukaemia are first referred to dentists, ophthalmologists, otorhinolaryngologists, orthopaedic or genito-urinary surgeons before the nature of their disease is recognized.

In the majority of elderly patients, and in some young ones, the classical picture of acute leukaemia with its abrupt onset, fever, gingivitis and stomatitis, purpura, and severe anaemia, is not found. As Gunz and Hough (1956) have emphasized, the onset of the disease in middle-aged and elderly patients is usually insidious, and slowly progressive symptoms of lassitude, anorexia and exertional dyspnoea have often been present for several months before medical advice is sought. In this group of patients the clinical history and physical signs may provide little suggestion of leukaemia, and tentative diagnoses of pernicious or aplastic anaemia, carcinomatosis, chronic bronchitis, cardiac failure and so forth have often been made before adequate investigation has revealed the true nature of the disease.

The fact that acute leukaemia may develop insidiously does not seem to be widely recognized, but it is undoubtedly a frequent mode of onset in the elderly and is by no means uncommon in younger patients. In this connection reference may be made to the existence of "preleukaemic" states before the onset of true acute leukaemia. Block, Jacobson and Bethard (1953) were able to make clinical and haematological observations on 12 patients (11 of them over the age of 40) who suffered from apparently non-leukaemic blood disorders for periods of 3 to 17 months before the development of frankly leukaemic changes. These patients showed peripheral cytopenias involving all cell series and had maturation arrest or hypoplasia of granulopoietic, erythropoietic and thrombopoietic tissues in the bone marrow. After variable periods, progression to typical primitive cell leukaemia took place. The clinical signs during such pre-leukaemic phases are those to be expected from the existence of pancytopenia, and include haemorrhages, anaemia and a tendency to infection. The spleen is sometimes palpable at this time and a diagnosis of primary hypersplenism or acquired haemolytic anaemia may be made. Occasionally splenectomy has been carried out during this period, with the subsequent unexpected development of acute leukaemia (Welch *et al.*, 1957). The appearance of maturation arrest with an active marrow during the preleukaemic phase is perhaps less common than the occurrence of an apparently typical aplastic pancytopenia which undergoes a transition to a fully leukaemic picture, sometimes passing through a temporary state of relatively normal marrow cellularity. Cases of this kind have been studied and reported on many occasions and are certainly not uncommon (Marchal *et al.*, 1944; Mallarmé, 1949; Meacham and Weisberger, 1954; Williams, 1955; Hayhoe, 1957). The relationship of aplasia to leukaemia is further discussed in Chapter 16, but it should be emphasized that, while aplastic phases may precede the onset of frank leukaemia or develop during the course of the disease, the great majority of patients with acute leukaemia have fully characteristic leukaemic changes in the bone marrow when they first seek medical attention.

Special symptomatology and correlated pathology

Enough has already been said to make it clear that the usual pattern of symptoms and signs at the time of onset of acute leukaemia is composed of a common background of anaemia, haemorrhage, stomatitis and fever, with a wide variety of possible additional features depending on organ or system involvement by localized infiltration, haemorrhage or infection. The changes to be found in the organs and tissues during the course of the disease will now be considered in more detail.

Lymph glands. Enlargement of lymph glands is not so conspicuous in acute leukaemia

as in the chronic lymphocytic form and is sometimes not detectable clinically. In many cases, however, either localized or generalized enlargement may be found. The glands are usually softer than those of Hodgkin's disease or chronic leukaemia and remain as discrete nodes, commonly from 1 to 3 cm. in diameter. Generalized lymphadenopathy is very often present in the lymphoblastic variety of acute leukaemia, but occurs sufficiently frequently in myeloblastic and monocytic forms of the disease to reduce its value as a point of differential diagnosis between the three. Nevertheless, there is no doubt, as *Forkner (1938) emphasized, that marked general enlargement of lymph glands or the* formation of substantial gland masses in the thorax, abdomen or elsewhere in acute leukaemia is very much more common in lymphoblastic leukaemia than in other forms. When severe infection, necrosis and ulceration in the mouth and pharynx exist, the cervical glands may be secondarily involved, and this is a finding often encountered in monocytic leukaemia and occasionally in other varieties of acute leukaemia, but general enlargement of lymph nodes is usually slight or absent in myeloblastic or monocytic leukaemia and in these conditions large gland masses very rarely form. Massive enlargement of lymph nodes, sufficient to cause severe obstructive symptoms, is not often encountered in acute leukaemia, but examples of respiratory obstruction from mediastinal glands in acute lymphoblastic leukaemia have been reported by Levison (1955) and Towers and Fountain (1955) and an illustrative case report will be found at the end of this chapter. Cases of this kind provide a link between lymphoblastic lymphosarcoma, with local tumour formation but without leukaemic changes in the blood and marrow, and the more usual form of acute lymphoblastic leukaemia. Gross lymph-node enlargement, most often mediastinal, may indeed precede the appearance of leukaemic changes in the blood in acute lymphoblastic as in chronic lymphocytic leukaemia, and it was to cases probably of this character that Sternberg (1915) first applied the name leukosarcoma (see also Chapter 16).

When observed at autopsy, enlarged lymph nodes of acute leukaemia appear macroscopically as soft, homogeneous, pinkish or greyish-white structures, remaining discrete from the surrounding tissues and usually from one another. Histologically the essential architecture of the lymph node can scarcely be discerned, having been replaced by a diffuse sheet of uniform cells of whichever primitive cell series is concerned, most often lymphoblasts, intermingled with more mature lymphocytes (Plate XXIII, Fig. 8). Occasional areas of haemorrhage may be seen, but there is little tendency to necrosis or to invasion of the capsule as in lymphoblastic lymphosarcoma. Nevertheless, it is often impossible to distinguish these two conditions from a study of lymph-node sections alone.

The spleen. Palpable splenomegaly is found in some 70 per cent of cases of acute leukaemia. It is greatest and most common in the lymphoblastic form, where the organ usually extends some 3 to 5 cm. below the costal margin and may, uncommonly, be grossly enlarged, reaching below the umbilicus and resembling the spleen of chronic granulocytic leukaemia. Enlargement of the spleen may develop very rapidly during the course of the disease, and there is some danger of spontaneous rupture. In most cases of lymphoblastic leukaemia, however, as in the other varieties of acute leukaemia, splenomegaly is slight or moderate. Pathologically the spleen shows diffuse infiltration with leukaemic cells and cannot easily be differentiated from the spleen of anaplastic or lymphoblastic lymphosarcoma, although there is perhaps less tendency to the formation

of circumscribed nodules, to the development of necrotic changes and to capsular invasion. In acute myeloid or monocytic leukaemias the primitive cells may show sufficient differentiation to allow certain recognition.

The liver. Slight or moderate enlargement of the liver is usually found in acute leukaemia at some stage of the disease, but gross hepatomegaly or severe disturbance of hepatic function is rare. Obstruction of biliary passages by enlarged lymph glands at the porta hepatis may lead to jaundice. At autopsy the liver is usually pale and soft and sometimes shows a fine macroscopically visible network of white or pale yellow infiltrates. Histologically, primitive cells are widely distributed throughout the sinusoids, but are usually more heavily concentrated in the portal tracts. The parenchymal liver cells are sometimes scattered by infiltrate but there is little sign of cell necrosis or destructive invasion and circumscribed nodules of leukaemic tissue are rarely present (Plate XXIII, Fig. 7).

The skin. The vast majority of skin manifestations of acute leukaemia are purpuric and nearly all patients show some haemorrhagic skin lesions during the course of their disease. The lesions range from scattered pin-point petechiae to large ecchymoses, and haemorrhagic bullae with necrosis, ulceration and secondary infection may sometimes occur. In lymphoblastic and myeloblastic leukaemia specific skin involvement by localized cellular infiltrates is quite uncommon, but in monocytic leukaemia a wide variety of specific skin rashes or tumours has been described, including diffuse maculo-papular eruptions, separated nodular lesions or plaques, and even generalized pustular rashes and exfoliative dermatitis (Hubler and Netherton, 1947). Histologically, these lesions show dense accumulations of monocytic cells in the dermis, and areas of haemorrhage are not uncommon, probably accounting for the bluish colour seen clinically in many of the lesions. Degos, Ossipowski and Morell (1956) emphasized the variability of cutaneous manifestations of leukaemia, and a survey of the many isolated case reports of skin lesions in the acute leukaemias confirms their view that it is impossible to make a firm diagnosis as to the type of leukaemia present, or even to be certain that the skin involvement is specifically leukaemic, from the clinical appearance of the skin alone (Plate XXII, Figs. 2 and 3).

Mucous membranes. Both non-specific and specific lesions are frequently found in the mucous membranes of the nose, mouth, and upper respiratory tract in all forms of acute leukaemia. Bleeding from the gum margins or from the nose, either spontaneously or after the minor trauma involved in brushing the teeth or blowing the nose, is a very common early manifestation of the disease and is often the first symptom. Petechiae appear on the oral mucosa and less commonly severe submucosal haemorrhage may occur, giving rise to extensive ecchymoses and haematoma formation and occasionally to ulceration, particularly if the patient has undergone dental extractions before the true nature of the disease is discovered.

Apart from these bleeding phenomena, the commonest oral complication of acute leukaemia is swelling and ulceration of the gums, often accompanied by an extension of the ulceration to the soft palate, the pharynx and other parts of the buccal mucosa. While lesions of this sort are to be found in many patients with acute leukaemias of the myeloblastic or lymphoblastic varieties, they are more common and severe in acute monocytic leukaemia, where diffuse hypertrophy of the gingivae may be so marked that the teeth become almost submerged in the s[illegible] spongy gums (Plate XXII, Fig. 1). Forkner

(1934) drew attention to the importance of this sign in the clinical differentiation of the forms of acute leukaemia, but although severe mouth lesions are certainly very common in acute monocytic leukaemia, comparable swelling and ulceration may occasionally be found in other varieties of acute leukaemia, so that the finding is suggestive rather than diagnostic. Histological examination of the hypertrophied gums shows oedema and perivascular infiltration with leukaemic cells, and when ulceration has occurred there are usually areas of haemorrhage, necrosis, and bacterial invasion with an inflammatory reaction containing many leukaemic cells.

Since the oral mucous membranes in acute leukaemia appear to have a lowered resistance to bacterial invasion as well as being subject to infiltration with leukaemic cells, ulcerative lesions may be of mixed origin and, even if specifically leukaemic initially, are readily invaded by spirochaetes, cocci and other mouth organisms. It is not therefore surprising that diagnoses of Vincent's angina, agranulocytic angina or even diphtheria have sometimes been made before further investigation has revealed the leukaemic process. It is a wise precaution to examine the peripheral blood of every patient with buccal ulceration, especially when there are signs of haemorrhage, before a local cause is accepted as primarily responsible.

A similar kind of infiltration and ulceration may involve the mucous membranes of the upper respiratory tract, particularly the larynx, and lesions will often be found on the epiglottis or vocal cords if sought. They occasionally provide the first symptoms of disease by causing hoarseness and an irritable dry cough, and when monilial infection in the mouth and pharynx arises either spontaneously or during antibiotic therapy, their presence probably predisposes to spread of the fungus into the respiratory tract.

Bones and joints. The existence of bone lesions in leukaemia, recognized since the early observations of Neumann (1878), has been shown by the widespread use of radiography in more recent years to be an extremely common feature in the acute form of the disease in children. Adults with acute leukaemia do not show bone involvement so often and it seems likely that the growing skeleton is particularly liable to be affected by destructive and irritant erosion and infiltration. Bone lesions have been found to be most frequent in lymphoblastic leukaemia with subleukaemic or aleukaemic peripheral blood. Baty and Vogt (1935) reported radiological bone changes, chiefly areas of diminished density proximal to the metaphyses of long bones, in 30 of 43 leukaemic children. Most of the affected children had aleukaemic leukaemias and in this series myeloblastic and lymphoblastic leukaemias were equally represented. In a later survey of 146 leukaemic children reported to have had bone lesions, Kalayjian, Herbut and Erf (1946) found that most were aleukaemic and that 140 were lymphoblastic and only 5 myeloblastic in type. Radiological bone changes were found by Dale (1949) in 29 of 40 leukaemic children, and here again the majority of the affected children had lymphoblastic leukaemia. These findings cannot be interpreted to mean that lymphoblasts infiltrate and damage the bony cortex or periosteum more avidly than other primitive cells; they probably merely reflect the relatively high incidence of lymphoblastic leukaemia as compared with other forms of acute leukaemia during the years of bone growth in childhood. The significance of the observation that bone changes are most common in aleukaemic leukaemia is also uncertain. It may be that aleukaemic patients, having less obvious and easily diagnosed leukaemia, are subjected to more thorough investigation revealing clinically undetected

of circumscribed nodules, to the development of necrotic changes and to capsular invasion. In acute myeloid or monocytic leukaemias the primitive cells may show sufficient differentiation to allow certain recognition.

The liver. Slight or moderate enlargement of the liver is usually found in acute leukaemia at some stage of the disease, but gross hepatomegaly or severe disturbance of hepatic function is rare. Obstruction of biliary passages by enlarged lymph glands at the porta hepatis may lead to jaundice. At autopsy the liver is usually pale and soft and sometimes shows a fine macroscopically visible network of white or pale yellow infiltrates. Histologically, primitive cells are widely distributed throughout the sinusoids, but are usually more heavily concentrated in the portal tracts. The parenchymal liver cells are sometimes scattered by infiltrate but there is little sign of cell necrosis or destructive invasion and circumscribed nodules of leukaemic tissue are rarely present (Plate XXIII, Fig. 7).

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body and so lead to confusion in diagnosis, since decreased density would be the expected change in leukaemia.

The bone changes in acute leukaemia result from the intense proliferation of primitive cells and the consequent tendency for sites of leucopoiesis to expand. In the adult there is relatively much more available space for this expansion, since haemopoiesis in the bone marrow is normally confined to the axial skeleton and large areas of yellow, fatty, marrow can be infiltrated and resume leucopoietic activity. This is, of course, not so in children, where most of the skeletal marrow is normally filled with haemopoietic tissue, and expansion of marrow leucopoiesis can only occur at the expense of cortical erosion and thinning. Probably the more common occurrence of bone lesions in acute leukaemia in children as compared with adults is partly explained by this fact, and partly by the increased susceptibility of growing bones to pathological influences. Griffiths (1955) has argued that subperiosteal ossification, as well as bone erosion, can be attributed to the need for increased marrow space, since the pattern of new bone formation suggests an attempt to form a new cortical layer with an expanded marrow cavity.

Involvement of joints in acute leukaemia is common enough clinically, although there have been few reports of pathological changes in clinically affected joints. Dresner (1950) described leukaemic infiltration of the synovial membranes and the presence of leukaemic cells in synovial fluid from an inflamed knee, and presumably similar changes are responsible for the pain and swelling often found in the joints in acute leukaemia. The possibility that children with the clinical picture of acute or subacute rheumatism or Still's disease, having pain and swelling of the larger joints, accompanied by anaemia and a haemic systolic murmur, and perhaps slight enlargement of the spleen and lymph glands, may in reality be suffering from acute leukaemia, must always be considered, and here again examination of the blood is essential. Since acute leukaemia is often aleukaemic, with few or no primitive cells in the peripheral blood, even an adequate blood examination may not reveal the diagnosis, and it is in such cases that the presence of radiological changes in the bones should direct attention to the strong likelihood that the apparent rheumatism is really leukaemic. A bone-marrow examination will then confirm the diagnosis. The very real difficulty in the differential diagnosis between acute leukaemia with joint involvement and rheumatic fever or Still's disease is well illustrated by a variety of reports (Beldridge and Awe, 1930; Poynton and Lightwood, 1932; Sutton and Bosworth, 1934; Bichel, 1948; Dresner, 1950), and in many of these cases the leukaemic process was not diagnosed until prolonged treatment with salicylates had failed to produce improvement and leukaemic changes had become manifest in the peripheral blood.

Central nervous system. That gross or microscopic infiltration of the central nervous system by primitive cells occurs in most cases of leukaemia has long been established, but clinical signs of neurological involvement are much less common. Trömner and Wohlwill (1927) found pathological lesions in the nervous system and meninges in 11 of 12 cases of leukaemia, although in 9 of these there were no clinical signs. Diamond (1934) found similar invasive lesions in the brain of each of 14 patients with various forms of leukaemia, but only 5 of these patients had developed neurological manifestations. Several comparable studies on larger numbers of patients have been reported, but in many of these the forms of leukaemia concerned have not been specified, and it is not easy to determine the relative incidence of clinical and pathological abnormalities in the different

bone lesions, but the possibility remains that a leukaemic process in which the delivery of primitive cells to the peripheral blood is held in check may be associated with a more locally damaging proliferation in sites of leucopoiesis.

The clinical and radiological features of bone lesions in acute leukaemia have been described in detail by Baty and Vogt (1935), Kalayjian, Herbut and Erf (1946), Silverman (1948), Dale (1949), Dresner (1950) and Griffiths (1955). The chief symptoms attributable to bone involvement in acute leukaemia are aching pains, sometimes very severe. They occur principally in the back or in the long bones, and since they may be the first symptoms of leukaemia the investigation of unexplained bone pains should invariably include a blood examination. When pains in the back or limbs occur in leukaemia, changes in the bones are almost always demonstrable. Silverman (1948) found lesions in all of 25 leukaemic patients who suffered from bone pains, whereas no lesions could be demonstrated in 34 patients without pains. Spinal deformity and neurological changes may follow vertebral collapse, and pathological fractures of long bones may occur, but the gross destruction necessary to produce such lesions is rarely found before the general leukaemic process is far advanced, and fractures and deformities are not common as initial symptoms.

Radiologically, the bone changes may be grouped as follows:

(a) *Disorders at the growing ends of long bones.* Zones of increased translucency at the metaphyses are very often found in leukaemic children, but these bands of decreased density are similar to those occurring in the same positions in many nutritional and debilitating diseases of childhood, such as the "Trümmerfeld zones" of scurvy and the translucent metaphyseal bands seen in chronic gastro-intestinal disorders. They are in no way pathognomic of leukaemia, but merely indicate the existence of a disorder of bone growth. They may, however, progress into distinct areas of bone erosion.

(b) *Destructive erosion and infiltration.* Destructive lesions may arise from a general expansion of the marrow cavity with progressive diffuse erosion of the trabeculae and thinning of the cortex, or a similar process may be confined to small local areas. The long bones are most often involved, but vertebrae, skull, pelvis and ribs may show demonstrable focal erosions (Plate XXIII, Figs. 3 and 4). Localized destructive lesions may merge with the areas of increased translucency at the shaft side of the epiphyseal line, but although focal erosions are commonly found in the metaphyses they may develop in almost any part of the skeleton. Either diffuse infiltration or the coalescence of small focal lesions may so weaken the cortex that pathological fractures occur. These are most common in the vertebrae, where wedge-shaped collapse ensues (Plate XXIII, Fig. 2). A concertina-like vertebral collapse may be seen when infiltration has weakened the bone structure without producing cortical fracture, and since the intervertebral discs are spared by the leukaemic process, the upper and lower surfaces of the softened vertebrae may become concave as a result of pressure from the discs.

(c) *Sub-periosteal new bone formation.* Cortical erosion due to leukaemic infiltration may stimulate ossification beneath the periosteum, a change most commonly seen near the metaphyses of long bones. The new bone may be formed parallel to the shaft or at right angles to it, and reactive ossification is sometimes so marked a feature that the appearance comes to resemble that of oesteogenic sarcoma or osteomyelitis.

(d) *Osteosclerosis.* Localized increase in bone density to X-rays is rarely seen in patients with acute leukaemia, but such an osteosclerotic change may sometimes affect a vertebral

and exudates are scattered over a hazy background in which the dilated and tortuous veins show pale perivascular sheathing due to infiltration with primitive cells (Plate XXII, Figs. 5 to 8). Rarely the whole fundus appears to have a pale orange or greenish tinge. While thrombocytopenia is obviously an important factor in producing retinal lesions, a high peripheral leucocyte count, with many primitive cells, and a low haemoglobin level are usually present when retinal changes are severe. Even when quite gross changes have occurred, a surprisingly complete return to normal may take place if a remission is induced, and the variability of fundal changes is a prominent feature in acute leukaemia. Full accounts of the ophthalmology of leukaemia have been written by Borgeson and Wagener (1929) and Goldbach (1933), and the subject was reviewed extensively by Forkner (1938). *The commonest symptom* associated with retinal or vitreous changes in leukaemia is failing vision, but although the sudden onset of blindness, resulting from large retinal haemorrhages, has sometimes provided the initial symptom of acute leukaemia, and the development of scotomata or narrowing of the visual fields may be a prominent feature, it should be emphasized that considerable retinal changes are often present in the absence of any significant ocular symptoms.

Apart from changes in the fundus, common lesions of the eyes in acute leukaemia include inflammation of the conjunctiva or sclera and subconjunctival haemorrhages (Plate XXII, Fig. 4), while leukaemic infiltration of the lachrimal glands or deposits within the orbit may sometimes be conspicuous and exophthalmos may be produced. Orbital tumours are among the commonest features of chloroma (see p. 287) but are not often seen in typical acute leukaemia.

The ears. Aural symptoms are not common in acute leukaemia but may occasionally be conspicuous. Haemorrhage into the semicircular canals, leading to Menière's syndrome of tinnitus, vertigo and nausea, followed by deafness, sometimes occurs, and leukaemic deposits may infiltrate the labyrinth, the middle ear or the auditory nerve, producing various degrees of deafness. The increased liability to infections shown by patients with leukaemia may lead to the development of acute otitis media.

The alimentary tract. Gastro-intestinal haemorrhage frequently occurs in acute leukaemia, usually as a result of thrombocytopenia rather than of specific infiltrated erosions, which are rare. Massive bleeding from the stomach or duodenum may occur, and present a difficult diagnostic problem, since patients with acute leukaemia may suffer also from gastric or duodenal ulcers, not necessarily related to the leukaemic state or to steroid therapy (Palmer, 1955). Severe haematemesis, especially when unaccompanied by generalized haemorrhagic phenomena, should therefore not be dismissed as due to leukaemic bleeding or erosions without careful investigation. Specific infiltrations of the stomach, ileum, appendix, caecum or rectum, with localized nodule formation and even ulceration and perforation, usually associated with submucosal or subserous haemorrhages, have occasionally been observed, and may give rise to acute abdominal symptoms (Boikan, 1931; Campbell, Henderson and Croom, 1936; Forkner, 1938). Although leukaemic lesions in the gastro-intestinal tract severe enough to cause symptoms are rare, infiltration with leukaemic cells is not uncommon at the anal muco-cutaneous junction and painful ulceration at this site may occur.

The genito-urinary system. Haemorrhagic and infective complications of acute leukaemia frequently involve the genito-urinary system, with attendant haematuria,

haematological varieties of leukaemia. Treating all leukaemias as a single group, Williams, Diamond and Craver (1958) reported a total of 276 cases with clinically detected neurological complications among 1,864 leukaemic patients. Intracranial haemorrhage was responsible for the neurological disturbance in 140 cases and 72 per cent of these patients had acute leukaemia. The prominence of intracerebral bleeding among acute leukaemic patients is certainly attributable chiefly to thrombocytopenia, and symptoms arising from intracranial haemorrhage are found at some stage of the disease, often terminally, in about 50 per cent of patients with the acute form of leukaemia, whatever its cytological variety. Thus, among 42 patients with acute leukaemia followed throughout the whole course of their disease, Wells and Silver (1957) found 22 with neurological manifestations, the majority being caused by cerebral haemorrhage.

Haemorrhages into the spinal cord or diffuse infiltrations with occasional nodular deposits of leukaemic cells in almost any part of the central nervous system, from the brain and spinal cord to the cranial and peripheral nerves, may give rise to a great variety of neurological symptoms and signs, and examples of hemiplegias, ocular, auditory, oculomotor and facial nerve palsies, pyramidal tract lesions, sensory and motor affections of peripheral nerves, and symptoms suggesting acute meningitis, encephalitis and transverse myelitis have all been reported (Schwab and Weiss, 1935; Forkner, 1938; Brandt, 1945; Leidler and Russell, 1945; Gilbert and Rice, 1957; Wells and Silver, 1957). The general pattern of neurological involvement does not appear to have changed substantially in recent years, although Sullivan (1957) reported an apparent increase in the incidence of raised intracranial pressure in children treated with steroids and antimitotic agents since 1956. She suggested that prolonged survival of treated children, who probably had a lower concentration of the chemotherapeutic agent in the cerebro-spinal fluid and central nervous system than elsewhere in the body, might allow cerebral involvement to become extensive, and noted that irradiating the entire skull usually relieved the phenomena of headache, vomiting, papilloedema, separation of skull sutures and raised spinal fluid pressure, which must presumably have been due to leukaemic infiltration of the brain and meninges. With the possible exception of this complication of controlling therapy, neurological manifestations, apart from those due to cerebral haemorrhage, are sufficiently rare in acute leukaemia for them to be reported as curiosities. An awareness of their existence is important, but they are not likely to be met frequently.

Herpes zoster has a particular association with malignant lymphomas and leukaemias, perhaps being precipitated by tumour involvement of some afferent part of the reflex arc (Craver and Haagensen, 1932), but it rarely develops in acute leukaemia.

The eyes. Ocular lesions are very common in acute leukaemia, the most important of them being retinal or vitreous haemorrhages developing during phases of severe thrombocytopenia. The retinal haemorrhages may be superficial or deep, flame-shaped or irregularly round, but most often have pale centres with red peripheral zones. This appearance is thought to be due to a central area of degenerate leucocytes arising as a result of embolism or of invasion by leukaemic cells. Accompanying the haemorrhages there is usually conspicuous dilatation of retinal veins, and small greyish-white nodules or diffusely outlined "cotton-wool" exudates are sometimes present. In the fully developed picture of acute leukaemic retinopathy there is extensive oedema of the fundus, with blurring of the disc outlines, almost resembling papilloedema, and numerous haemorrhages

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The genito-urinary system. Haemorrhagic and infective complications of acute leukaemia frequently involve the genito-urinary system, with attendant haematuria,

menorrhagia or symptoms of nephritis or cystitis. Specific infiltration of the genito-urinary tract with primitive cells can usually be demonstrated on histological examination, the kidneys being nearly always involved (Plate XXIII, Fig. 6), the bladder less often and the genital organs in perhaps a third of all cases. Symptoms directly attributable to leukaemic infiltration of these organs are not often seen, but occasionally pain in the region of the kidneys, ovaries or testicles, or obstructive swelling of the prostate may have been prominent during life, when leukaemic deposits with no evidence of infection or serious haemorrhage at these sites is found at autopsy. Early case reports of genito-urinary involvement, including those of Gwyn (1930), Mach (1931) and White and Burns (1931), were reviewed by Forkner (1938), and more recent reports of isolated cases have added little to the general picture. Lucia and his associates (1952), in an extensive study of visceral involvement in primary neoplastic diseases of the reticulo-endothelial system, obtained data on the frequency of urogenital tract infiltration in 49 patients with acute leukaemia. Of 8 patients with acute stem-cell leukaemia, 7 showed infiltration of the kidneys, 2 of the uterus, 2 of the ovaries and 2 of the testes. In 14 patients with monocytic leukaemia, the kidneys were infiltrated in 11, the bladder in 5, the uterus and ovaries in 1, the testes in 8 and the prostate in 7. In 17 patients with lymphoblastic leukaemia, the kidneys were involved in 15, the bladder in 7, the uterus in 2, ovaries in 2, the testes in 5 and the prostate in 3. In 10 patients with myeloblastic leukaemia, the kidneys were infiltrated in 5, the bladder in 3, the uterus in 2, ovaries in 1 and the prostate in 2. The varieties of acute leukaemia do not therefore exhibit striking differences in the frequency of urogenital involvement, but the authors observed that the myeloblastic form tended to produce the least prominent visceral deposits, while the largest leukaemic nodules were usually found in the acute monocytic form. Nevertheless, massive invasion of the pelvic organs does sometimes occur in forms of acute leukaemia other than monocytic (Winkelstein, Kato and Sharnoff, 1955).

Respiratory system. Infiltration in the respiratory tract, localized anywhere from larynx to lung parenchyma, may rarely be of sufficient degree to cause prominent symptoms. Extensive lesions of this nature are distinctly uncommon, but scattered miliary foci of leukaemic infiltration, not gross enough to have provoked obvious pulmonary symptoms, are frequently found at autopsy (Plate XXIII, Fig. 5). Nathan and Sanders (1955) reviewed a total of 59 consecutive autopsied cases of acute leukaemia and found definite leukaemic involvement of the lung parenchyma in 14 of them. Specimens in which leukaemic cells were confined to the lumen of blood vessels were excluded. The infiltrations were most commonly found invading the alveolar septa. The cellular accumulations were sometimes dense enough to compress the capillaries and even the alveoli, but were generally distributed in separated foci, each a few millimetres or less in diameter. A second common site of infiltration was in the neighbourhood of small bronchioles and blood vessels, while less frequently subpleural foci of primitive cells were observed. Areas of pulmonary infarction secondary to vascular obstruction by leukaemic cells were not observed in this case series, but were earlier reported by Joachim and Loewe (1927) and Fiessinger and Fauvet (1941). Leukaemic infiltration in the lungs is often not visible on radiographs, but miliary shadows may be found, and if the lesions become extensive and severe, impairment of pulmonary function is likely to occur, with the possible development of the "alveolar-capillary block" syndrome. Nathan and Sanders did not differentiate between the cytological

varieties of acute leukaemia, but in the course of their survey of visceral lesions Lucia and his associates (1952) found the lungs involved in 4 of 8 stem-cell leukaemias, in 12 of 14 monocytic, in 12 of 17 lymphoblastic and in 5 of 10 myeloblastic leukaemias. The overall incidence of pulmonary involvement in acute leukaemia therefore appears to be between 30 and 65 per cent, while lesions are least common and conspicuous in the myeloblastic form.

Myocardium and pericardium. Infiltration of the myocardium with leukaemic cells is found, *post mortem*, in about 60 per cent of patients with acute leukaemia (Plate XXIII, Fig. 9), but symptoms attributable to such infiltration are rare (Forkner, 1938; Lucia *et al.*, 1952). Invasion of the pericardium is much less common, although an increase in pericardial fluid is frequently found at autopsy. Signs of cardiac failure may result from anaemic anoxia of the myocardium in patients with prolonged severe anaemia, and this condition may sometimes be encountered in the terminal stages of acute leukaemia, especially in elderly patients.

Findings in the blood and bone marrow

The blood. The anaemia and bleeding tendency of acute leukaemia have been discussed already in Chapter 11, and it has been seen that, while both are commonly severe at some stage of the disease, their pathogenesis is uncertain. Morphologically, the red cells are usually normocytic and normochromic even when their numbers are greatly reduced, but macrocytosis may sometimes be conspicuous, and variations in size and shape are not uncommon and may occasionally be striking. There may be polychromasia and reticulocytosis when red cell regeneration is active, and a few normoblasts are usually present. During the early stages of remission these features are often exaggerated, and a significant increase in reticulocytes is an important sign of commencing remission. Persistently high numbers of reticulocytes and normoblasts are unusual in the more typical forms of acute leukaemia, but are found in erythro-leukaemias, where the erythroblast series of cells appears to be involved in a parallel proliferative process to that of the leucopoietic tissues.

During phases of thrombocytopenia, when platelet numbers have fallen to between 10,000 and 100,000 per cu. mm., the morphology of the few platelets to be seen in blood-smears is often abnormal, with irregularities of size, shape and staining. Large platelets, 3 to 5 μ in diameter, with sharply demarcated hyalomere and granulomere, often predominate when the platelet count begins to rise at the onset of remission, and the presence of these characteristic young platelets is a further indication that remission is likely to develop.

In all forms of acute leukaemia the total leucocyte count in the peripheral blood may vary between very wide extremes. In the majority of cases, levels between 20,000 and 50,000 leucocytes per cu. mm. are found during the early stages, but in a substantial minority, amounting probably to some 30 per cent of all cases, the initial leucocyte counts are subnormal or within the normal range and in exceptional cases figures up to 500,000 or more may be reached. When high counts are found, the predominating cells are leucocyte precursors, chiefly the most primitive forms of the cell series concerned, either myeloblasts, lymphoblasts or early monocytes. The blood picture with lower total leucocyte counts may be subleukaemic or aleukaemic (see Chapter 2), but it is rare for an entirely

TABLE IV (and see Plates XIX, XX and XXI)

	Acute Myeloblastic Leukaemia	*Acute Lymphoblastic Leukaemia*	*Acute Monocytic Leukaemia*
Romanowsky staining (See also Plates II, III and IV)	Chief cells are myeloblasts; diam. 10–20 μ; nuclear-cytoplasmic ratio variable, but nucleus usually large, with smooth leptochromatic chromatin, finely stranded or stippled, and containing 2 to 5 nucleoli of a pale blue colour. Neither the nuclear membrane nor the nucleolar outlines are usually strongly marked. Cytoplasm is moderately deeply basophilic and sometimes has a clearer zone at one side of the nucleus. Rarely there is cytoplasmic vacuolation and a spongy or reticular appearance. There are commonly a few, and sometimes many, promyelocytes, having similar characteristics to myeloblasts, but possessing variable numbers of coarse, deep red "azurophil" granules, and sometimes a few specific granules. Both myeloblasts and promyelocytes in acute leukaemia may show irregular indentation and folding of the nucleus—"paramyeloblasts and parapromyelocytes", and the nucleus may appear lobulated—"Rieder cells". Auer bodies may be seen in the cytoplasm. Myelocytes and metamyelocytes are uncommon, but mature polymorphs and lymphocytes are often present in small numbers.	Chief cells are lymphoblasts; very similar in size and general appearance to myeloblasts, but with coarser, more deeply staining nuclear chromatin, and fewer nucleoli. The nuclear membrane and the nucleolar outlines are more conspicuous than in the myeloblast. Indentations of the nucleus or Rieder cell formation are not uncommon. The cytoplasm is usually more scanty than that of myeloblasts and the cytoplasmic edge is often ragged in places, with small free fragments of cytoplasm detached from the parent cell. Vacuolation is sometimes very marked and the detached cytoplasmic fragments may contain vacuoles. Occasional prolymphocytes, intermediate between lymphoblasts and mature lymphocytes, may be present, but they are rarely numerous. A very occasional promyelocyte or later granulocyte precursor cell may be detected, liberated presumably as a result of extensive marrow infiltration and disorganization, but such cells are very few in numbers. Mature lymphocytes and a few polymorphs are usually present.	Chief cells are promonocytes; usually rather larger than myeloblasts—15–25 μ diam.—and with irregularly twisted, folded and indented nuclei, occupying a smaller proportion of the whole cell than do the nuclei of myeloblasts or lymphoblasts. The nuclear chromatin is generally smooth and fine, but irregular in staining and suggesting the open "basket-work" pattern of the mature monocyte nucleus. Nucleoli are faint or absent. The abundant cytoplasm is only lightly basophilic, is often hazy, and frequently shows marked irregularities of outline and extensive vacuolation. Auer bodies may be seen, and there are usually very fine scattered, azurophil granules. Monoblasts, closely resembling myeloblasts or paramyeloblasts, may be present, often in considerable numbers, while fully mature monocytes are not uncommon. In the pure, Schilling, type of acute monocytic leukaemia, all or nearly all precursor cells are of the monocytic series, but in the mixed myelo-monocytic or Naegeli form, granulocyte precursors including promyelocytes and myelocytes are numerous, although promonocytes usually still predominate.
Peroxidase reaction (See also Plate XIV)	Variable degree of cytoplasmic positivity. More mature myeloblasts and all promyelocytes show some positive granules, and these are sometimes coarse and numerous. When the predominant cells are very immature the reaction may be found in only a few cells or even in none, but some granules are detectable in most cases of acute myeloblastic leukaemia.	Cells of the lymphocytic series are invariably negative, and the only positive reactions encountered are in mature polymorphs and the very rare granulocyte precursors sometimes present. The general picture is always one of overall negativity.	The reaction shows much variation, being usually completely negative, but sometimes irregularly positive, with some promonocytes showing no granules but others showing a scattering of fine positive granules. Granulocyte precursors in the Naegeli form show the expected more or less coarse positivity.

Sudan black B (See also Plates XII and XIII)	Positivity generally parallels the peroxidase reaction, but is occasionally present when no peroxidase granules have been detected. In addition to coarsely positive sudanophilic granules in promyelocytes and later myeloblasts, most primitive cells show fine juxtanuclear rods and dots, *presumably due* to cell organoids.	Apart from fine granules in the juxtanuclear region, lymphocytic precursor cells are uniformly negative, and the results of Sudan-black staining again tend to parallel those of the peroxidase reaction.	Promonocytes are variably positive, but usually show small finely scattered discrete sudanophilic granules, while mature monocytes show coarser granules. Monoblasts resemble other primitive cells in having faintly positive dots close to one side of the nucleus.
Glycogen (P.A.S. reaction) (See also Plates VIII, IX and X)	Faint granular positivity is detectable in promyelocytes and a more definite reaction in any later granulocytes, but myeloblasts are negative, with no PAS-positive granules and only occasionally a faint pink tinge in the cytoplasm.	Many lymphoblasts show strong and coarse positivity, with large discrete cytoplasmic granules, and sometimes blocks of PAS-positive material 2–4 μ in diam., in the cytoplasm or overlying the *nucleus*. Detached fragments of cytoplasm may contain glycogen.	Finely scattered granules of moderate intensity in many promonocytes and a similar but coarser reaction in mature monocytes. Monoblasts have very few fine granules or are negative.
Alkaline phosphatase (See also Plates XV and XVI)	Primitive cells are negative, and later cells of the granulocyte series, including mature polymorphs, are also strikingly negative.	Lymphoblasts and lymphocytes are negative, but the few mature polymorphs usually present are very strongly positive, sharply contrasting with the polymorphs of myeloblastic leukaemia.	Monoblasts and promonocytes are negative. Mature polymorphs have variable positivity, being strongly positive when the overall picture is predominantly monocytic, but less positive when a mixed myeloid reaction is present.
Feulgen reaction (See also Plates VI and VII)	This staining method reveals clearly the presence of 2–5 nucleoli and the leptochromatic nuclear pattern, and is particularly helpful in revealing the primitive nature of micromyeloblasts which may resemble lymphocytes in Romanowsky preparations.	The nucleoli are clearly shown as 1 or 2 large unstained areas in the leptochromatic nucleus, with heavy nucleolus-associated chromatin and prominent nuclear membrane.	The nuclear pattern seen with Romanowsky stains is accentuated and occasional nucleoli may be seen in promonocytes. Monoblasts resemble myeloblasts.
Phase-contrast microscopy (See Plates XVII and XVIII)	Myeloblasts are well rounded with several nucleoli and with mitochondria scattered throughout the cytoplasm.	Lymphoblasts have conspicuous nucleoli, and mitochondria are clumped together close to one side of the nucleus.	Cell outlines often irregular, nucleoli inconspicuous, and motility a prominent feature.

aleukaemic picture, with no primitive cells at all, to be found, and when this situation exists a diagnosis cannot be made from examination of the peripheral blood. The presence of anaemia and thrombocytopenia without diagnostic leucocyte changes should lead to the study of aspirated bone marrow, however, and the marrow cytology should prove conclusive.

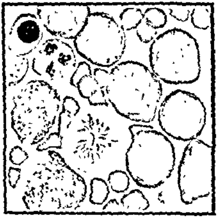
The various forms of differentiated leucocytes are easily distinguished one from another, but their immature precursors have a general similarity in appearance, and since in many cases of untreated acute leukaemia more than 80 per cent of the peripheral blood leucocytes are of uniform immature type with few cells of an intermediate stage in development, cytological classification often presents considerable difficulty. Nevertheless, by careful study of Romanowsky preparations, supplemented by cytochemical stains and phase-contrast microscopy, differentiation of the cytological varieties of acute leukaemia can usually be made. In Chapter 5 some account has been given of the cytology and cytochemistry of leukaemic cells as compared with normal leucocytes and reference has been made to certain points of difference between myeloblasts, lymphoblasts and monoblasts. The chief features helpful in differentiating the forms of acute leukaemia are summarized in Table IV. By the use of these criteria the great majority of cases of acute leukaemia can now be classified as myeloblastic, lymphoblastic or monocytic, but when full cytochemical studies cannot be made and in rare cases when the leukaemic cells are unusually primitive and difficult to classify, the term "stem-cell" leukaemia may be applied.

The bone marrow. Post-mortem examination of the bone marrow of patients with acute leukaemia reveals widespread hyperplasia, usually diffuse, but sometimes irregularly nodular, extending throughout the whole marrow cavity of axial and limb skeleton alike. Macroscopically, both the "red" and "yellow" areas of marrow are uniformly replaced by pinkish-grey leucopoietic tissue. Occasionally nodules of more creamy hue, due to particularly intense proliferation, areas of deep red haemorrhage, and sometimes white or yellow infarcts may be seen (Plate XXIII, Fig. 1), but the general picture is one of uniform change. Microscopically the normal erythropoietic and granulopoietic cells appear relatively very few in numbers, although they may in fact be absolutely increased, and the overwhelming majority of cells are primitive, immature, leucoblasts of whichever cell series is involved. Megakaryocytes are few in number.

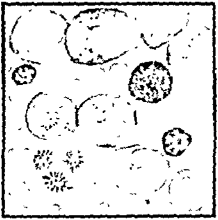
Aspiration of bone marrow by puncture of the sternum, iliac crest or a vertebral spine during life is a most valuable procedure for establishing the diagnosis in aleukaemic cases and in differentiating leucopenic acute leukaemia from idiopathic thrombocytopenic purpura, agranulocytosis and aplastic anaemia. In most cases, whatever the peripheral-blood findings, the marrow aspirate proves to be highly cellular and composed predominantly of primitive cells. The nature of the dominant cells in the marrow in each of the forms of acute leukaemia is closely similar to that already described for the peripheral blood of frankly leukaemic cases, and the criteria for cytological differentiation of primitive cells apply whether the cells are derived from blood or marrow. Although "blast" cells commonly constitute over 80 per cent of the cells in bone-marrow smears from untreated acute leukaemia, poorly cellular specimens without a great proportion of primitive cells are sometimes obtained. This may be due to irregular involvement of the bone marrow, when a second puncture at a different site will yield a cellular, diagnostic specimen, or it

PLATE XIX

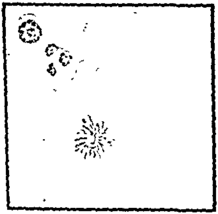
CYTOLOGY AND CYTOCHEMISTRY OF ACUTE MYELOBLASTIC LEUKAEMIA



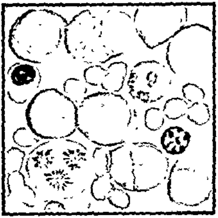
1. May-Grünwald-Giemsa stain



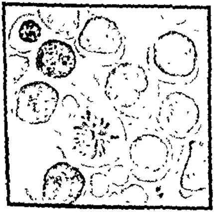
4. Sudan black B stain



2. Feulgen reaction



5. Peroxidase reaction



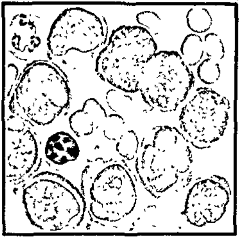
3. Periodic acid-Schiff reaction

6. Alkaline phosphatase reaction

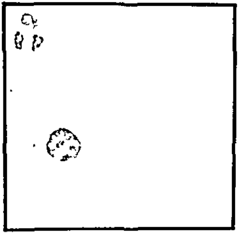
To face p 224

PLATE XX

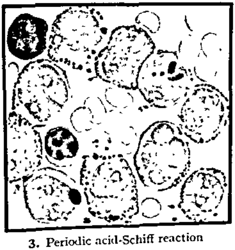
CYTOLOGY AND CYTOCHEMISTRY OF ACUTE LYMPHOBLASTIC LEUKAEMIA



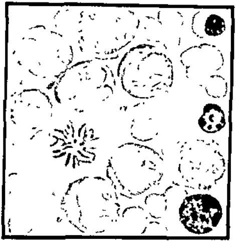
1. May–Grunwald–Giemsa stain



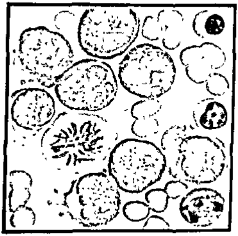
2. Feulgen reaction



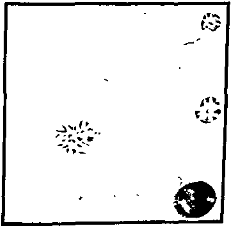
3. Periodic acid-Schiff reaction



4. Sudan black B stain



5. Peroxidase reaction

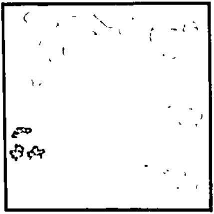


6. Alkaline phosphatase reaction

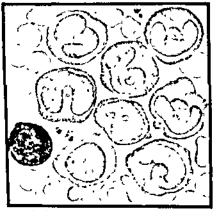
PLATE XXI

CYTOLOGY AND CYTOCHEMISTRY OF ACUTE MONOCYTIC LEUKAEMIA

1. May–Grunwald–Giemsa stain

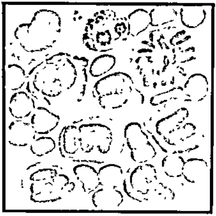


2. Feulgen reaction

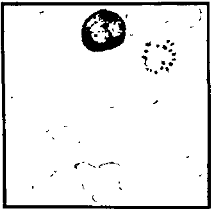


3. Periodic acid-Schiff reaction

4. Sudan black B stain



5. Peroxidase reaction



6. Alkaline phosphatase reaction

PLATE XXII

ACUTE LEUKAEMIA—CLINICAL FEATURES

1. Gingival swelling in acute monocytic leukaemia. In places the teeth are almost completely submerged. (Case No. 3.)

2 and **3.** Views of an extensive maculo-papular rash, dusky red in colour, in acute myeloblastic leukaemia. (Case No. 4.)

4. Subconjunctival haemorrhages as the presenting feature in acute lymphoblastic leukaemia.

5, 6, 7, 8. Retinal photographs showing haemorrhages and exudates, venous engorgement, and fundal oedema with blurring of the disc margins, resembling papilloedema, from patients with acute leukaemia.

PLATE XXII

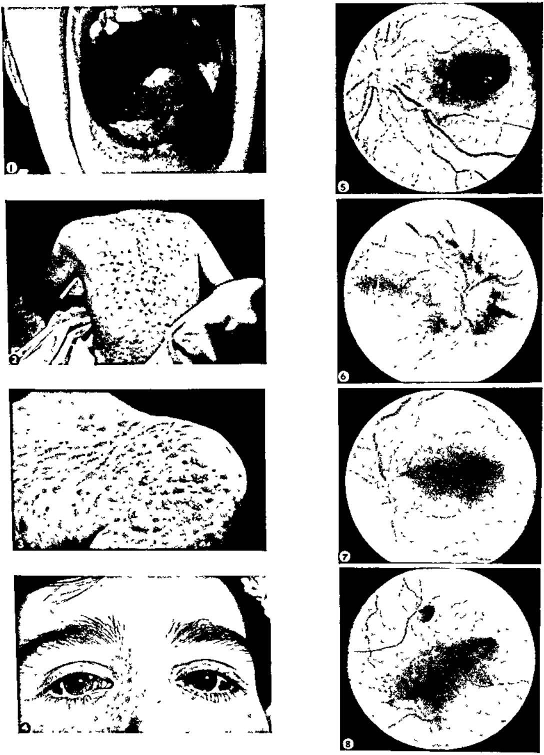


PLATE XXII

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5, 6, 7, 8. Retinal photographs showing haemorrhages and exudates, venous engorgement, and fundal oedema with blurring of the disc margins, resembling papilloedema, from patients with acute leukaemia.

may be because the leukaemic process is in an aplastic phase, when diagnosis may remain uncertain until frank leukaemic proliferation develops.

Differential diagnosis from non-leukaemic diseases

Once the possibility of acute leukaemia has been envisaged, examination of the peripheral blood and, if necessary, of the bone marrow, will enable the diagnosis to be firmly established in nearly every case. The problems of differential diagnosis in acute leukaemia are therefore few, but it is important to emphasize the variable symptomatology that may occur in the early stages and the superficial resemblance sometimes given to other disease states. As we have already seen, predominant symptoms and signs may arise in many different localizations, and the primary non-leukaemic diseases which may be simulated include Vincent's angina, diphtheria, osteomyelitis, rheumatic fever, pelvic, abdominal or mediastinal tumours, acute septic infections, miliary tuberculosis, various neurological states such as meningitis and myelitis, and a wide range of skin diseases. Provided the physician is aware that acute leukaemia enters into the differential diagnosis of these diseases, its existence can usually be confirmed or excluded without delay or difficulty by haematological examination.

A few conditions may present more difficulty in differential diagnosis in that the blood picture as well as the clinical findings may be confused with those of acute leukaemia. Infectious mononucleosis, especially the anginose form, may resemble leukaemia clinically, ✓ and the "glandular fever cells" of the peripheral blood can be mistaken for primitive cells by the inexpert, although they rarely show nucleoli and usually have coarsely stranded or clumped nuclear chromatin quite unlike that of the immature leucocytes of acute leukaemia. In glandular fever, however, there is usually no haemorrhagic tendency or anaemia, the platelet and haemoglobin levels are normal, and the total leucocyte count does not often exceed 30,000 to 40,000 cells per cu. mm. The Paul-Bunnell test for heterophil agglutinin active against sheep red cells is positive in many cases of infectious mononucleosis, and, most conclusive of all, the bone marrow does not show extensive infiltration with abnormal cells in any way comparable with the marrow replacement by blast cells found in acute leukaemias.

The similarities between aleukaemic acute leukaemia and aplastic anaemia, hypersplenic pancytopenia, idiopathic thrombocytopenic purpura and agranulocytosis may be very striking so far as the clinical picture and peripheral-blood state are concerned, but

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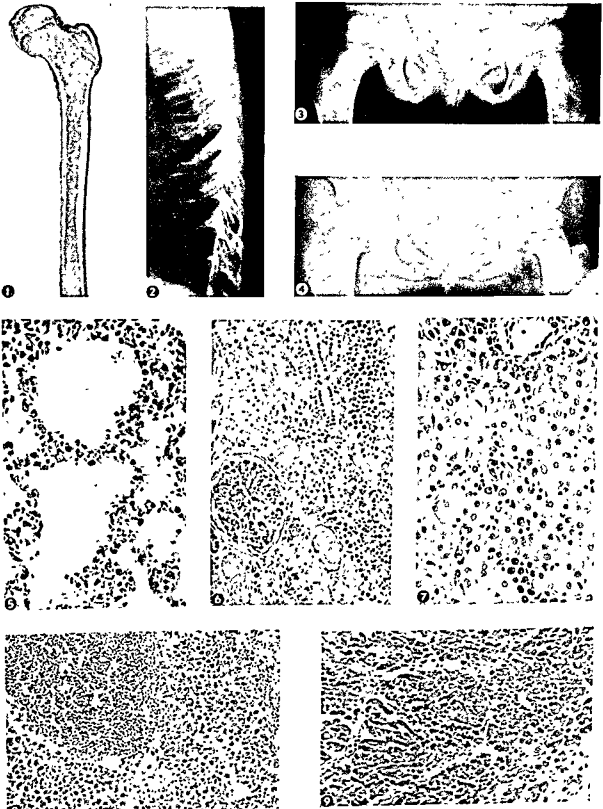
PLATE XXIII

ACUTE LEUKAEMIA—SKELETAL AND HISTOLOGICAL FEATURES

1. Areas of infarction and haemorrhage in the hyperplastic marrow of acute leukaemia.
2. Vertebral osteoporosis and collapse in acute lymphoblastic leukaemia.
3. Rarefaction and erosions at inner border of femoral necks and beneath the ischial periosteum in acute leukaemia.
4. Radiograph of same patient, showing healing of the bony lesions three months later during remission induced and maintained by aminopterin.
5, 6, 7, 8 and 9. Infiltration with acute leukaemic cells in lung, kidney, liver, lymph gland and myocardium. (× 100, × 50, × 100, × 50, × 50)

PLATE XXIII



tinic acid hydrazide presumably because it is more difficult for the organism to make enzymatic adaptations to two modes of metabolic interference than to one. Use of combinations of steroid hormones with antimetabolites might be expected, in a similar fashion, to produce more complete and lasting remissions. Experience in clinical practice has lent guarded support to this supposition. Employing folic acid antagonists in combination with ACTH or cortisone administered either concurrently or in alternating short courses, Marie and his co-workers (1951) and later Bernard and Mathé (1952) reported very good responses in children with acute leukaemia. There was an apparent synergistic effect of the drugs and less toxicity than with either agent alone. Magnin (1953), Kelty and Beard (1953) and Dameshek (1954) agreed as to the advantages of such combined therapy, reporting remissions in as many as 90 per cent of their cases. The patients treated have mostly been children, whose susceptibility is generally greater than that of adults, and since the folic acid antagonists alone are rarely of value in adult cases the combination seems unlikely to be very profitable in such patients. The slight potentiating action of azaserine on 6-mercaptopurine (Burchenal, 1954) has already been described. Mercaptopurine and amethopterin do not appear to act synergistically in leukaemic patients (Farber, 1954). Concurrent combination therapy has not yet been employed in large series of cases with all possible combinations of drugs and the erratic natural course of acute leukaemia makes it difficult to draw firm conclusions from small numbers of cases. The place of such treatment has not, therefore, yet been clearly established and, in general, the available agents have most often been used in sequence.

A further point to be considered in deciding which of the potentially effective drugs to use is the time required for their effect to become manifest. The antimetabolites usually require from 2 to 3 weeks before their administration leads to remission, whereas the steroid hormones, and blood transfusion, when effective, produce striking improvement commonly within a week.

Recommendations for general clinical management of acute leukaemia in children and in adults have been given, in the light of all these observations and considerations, by Burchenal (1954), Hayhoe and Whitby (1955), Burchenal and Ellison (1956) and Farber *et al.* (1956). In the first place simple transfusion alone may lead to remission of greater or less extent in from 20 to 30 per cent of patients, the outcome of treatment being usually predictable within a week, with a fall in the proportion of primitive cells in the blood and bone marrow and an increase in reticulocytes and platelets in those who respond. Second remissions may sometimes be induced by this means. Hormonal or other chemotherapeutic methods are more often and more fully effective, however, and treatment is therefore best initiated with one of these agents, supplemented by blood transfusion if there is moderate or severe anaemia. The choice of agent varies according to the type of primitive cell present, whether myeloblast, lymphoblast or monoblast, and is influenced also by the height of the peripheral leucocyte count, the clinical severity of the condition and whether the patient is an adult or a child.

Lymphoblastic leukaemias, whether aleukaemic or not and whether in child or adult, are most likely to respond quickly to the steroid hormones, and prednisone, combined with whole blood transfusion, is recommended as the initial mode of attack in patients with this form of leukaemia who are acutely ill. While this treatment is very effective in producing a rapid improvement, remissions induced by prednisone have generally a shorter

unless the leukaemia has entered an aplastic phase, marrow examination should reveal the great preponderance of primitive cells and leave the diagnosis in no doubt.

Acute exacerbation of chronic leukaemias may come to resemble acute leukaemia, but there is seldom a uniform picture of primitive cells in blood and marrow, the usual finding being one of mixed cell proliferation involving many later leucocyte precursors as well as blast cells.

The special problems of leukaemoid reactions, when non-leukaemic diseases stimulate leucopoietic proliferation and reversion to immaturity are discussed in Chapter 16.

Although patients with acute leukaemia do not often remain incorrectly diagnosed for long, the percentage initially diagnosed on clinical grounds as suffering from non-leukaemic diseases is surprisingly high. Thus in a recent survey of the records of a children's hospital, Oehme (1957) found that only 10 of 98 cases of acute leukaemia admitted between 1944 and 1955 had been correctly diagnosed by the admitting physician. In 6 patients the possibility of leukaemia was suspected, in 12 no diagnosis was made, and the remaining 70 patients were erroneously diagnosed as follows: haemorrhagic diathesis 13, contagious diseases (infectious mononucleosis, influenza, meningitis, mumps, poliomyelitis, sepsis, scarlet fever) 13, primary anaemia 11, rheumatism and endocarditis 10, gastro-intestinal diseases 6, tuberculosis 4, Hodgkin's disease 3, panmyelopathy 3, osteomyelitis 3, bronchopneumonia 1, glossitis 1, buccal cellulitis 1, nephritis 1. This range of incorrect diagnoses very well illustrates the difficulties of early clinical recognition of acute leukaemia and points to the importance of careful blood examination before any of these conditions, capable of confusion with leukaemia, is accepted as the primary cause of illness.

Treatment of Acute Leukaemia

The general principles of chemotherapy and supportive treatment and the modes of action, dosages and possible clinical applications of a wide variety of therapeutic agents have been discussed earlier in Chapters 10 and 11. It now remains to marshal views on the best and most appropriate methods of use of the drugs at present available in the management of acute leukaemias.

The compounds of proven value in inducing remissions in acute leukaemia include antimetabolites such as 6-mercaptopurine and the antagonists of folic acid and the steroid hormones corticotrophin, cortisone, prednisone, prednisolone and related substances. In addition the special role of blood transfusion and the occasional value of desacetylmethylcolchicine have to be considered. In the case of every one of these agents resistance to treatment develops sooner or later and the remissions induced rarely last more than a few months. Although there does not appear to be any cross-resistance between the groups of anti-leukaemic substances referred to, and response to an agent of one group may be achieved after resistance has developed to a member of another group, nevertheless successive remissions grow decreasingly full and prolonged. Much attention has been devoted to the use of combinations or alternations of agents on the supposition that the acquisition of drug resistance by the leukaemic cell parallels to some extent the development of resistance by bacteria to antibiotics and chemotherapeutic drugs. Thus the development of streptomycin resistance by the tubercle bacillus may be prevented or deferred if the antibiotic is used in combination with para-aminosalicylic acid or isonico-

prevent rapid deterioration during the transition period. When resistance again develops, prednisone may be used alone, demecolcine may be tried, or various agents may be used in combination.

In adults, since folic acid antagonists are of little value initially, 6-mercaptopurine is at present the antimetabolite of choice, but when a refractory state emerges it is worth trying amethopterin, since sensitivity to this agent sometimes appears to develop during the course of treatment with mercaptopurine.

Continuous, rather than intermittent, therapy with the chosen antimetabolite appears to give the most prolonged and satisfactory remissions and is therefore generally to be preferred even when the blood and marrow findings have returned substantially to normal. During either the preliminary stages of treatment with high dosages of antimetabolites or prednisone, or during prolonged maintenance therapy with smaller dosages, indications may arise for temporary cessation of treatment. We have already noted that the development of an aplastic state provides such an indication, and one should not hesitate to perform repeated marrow punctures to determine the extent of aplasia, leukaemic infiltration or normal regeneration duing this period, so that therapy can be stopped or restarted at appropriate times. Other indications for reducing dosage or stopping treatment are seriously toxic side-effects of the steroids, with severe fluid retention and oedema formation, hypertension, diabetes, hypokalemia or psychosis. These effects are quite uncommon in patients with acute leukaemia, even when large doses of prednisone are given, and the less serious signs of hypercorticism, with rounding of the face and mild fluid retention, do not necessitate stopping treatment. Reduction of salt intake and dietary supplements of potassium chloride, 3 to 6 gm. daily, help to reduce the incidence of side-effects. In the case of folic acid antagonists, doses within the ordinary therapeutic range often cause some loss of hair and occasionally diarrhoea or stomatitis, but treatment need not be stopped unless the effects are marked.

Another danger associated with aggressive chemotherapy is the development of renal failure due to hyperuricaemia following very rapid breakdown of cells. Dosages should be adjusted to avoid excessively rapid falls in the leucocyte levels and fluid intake should be high during the period of risk.

Illustrations of the use of hormones and antimetabolites in sequence or in combination will be found in the case reports at the end of this chapter.

The use of supportive and symptomatic measures for the control of anaemia, haemorrhage and infections occurring during the course of acute leukaemia has been discussed previously in Chapter 11.

Course and prognosis

The erratic natural course of acute leukaemia, with irregular fluctuations in the rate of deterioration, occasional spontaneous remissions, and complicating events such as haemorrhage and infection which may be serious enough to prove fatal, makes prognosis extremely difficult in an individual case and gives a wide scatter to collected survival figures. Nevertheless, some impression of the general effect of specific therapy may be gained from survival figures now available, although considerable differences appear to exist between the reported experiences of different observers. Burchenal and Ellison (1956) found that a substantial increase in survival time of children with acute leukaemia followed

duration than those induced by antimetabolites, and it is, moreover, desirable to avoid the development of resistance to steroids in the early stages of treatment, so that they may still be available and effective for use in later acute relapses and to cover periods of transition from one antimetabolite to another. For these reasons an attempt should be made to produce control by antimetabolites as soon as possible, and a suitable compound, amethopterin or some other folic acid antagonist in children and a purine antagonist such as 6-mercaptopurine in adults, may be given concurrently with the prednisone and transfusion from the start of treatment. During the first 2 or 3 weeks of this regime changes may be expected to occur in the peripheral blood and bone marrow, with sharp decrease in the numbers and proportion of primitive cells and often the temporary appearance of an aplastic or hypoplastic marrow picture with a peripheral pancytopenia, followed by the reappearance of normal haemopoiesis in the marrow and a steady rise in haemoglobin, red cells and platelets, with reticulocytosis and a return of the leucocyte count towards normal total and differential figures. At this stage the prednisone may be gradually tailed off and an attempt made to maintain the remission by maintenance doses of the chosen antimetabolite. When the aplastic, pancytopenic phase is marked, with few cells in marrow aspirates and leucocyte count below 2,000 or 1,500 per cu. mm., temporary discontinuance of antimetabolite therapy is advisable to avoid the danger of irreversible marrow failure, and here the best guide in deciding whether to stop antimetabolite treatment or not is given by the marrow state rather than the peripheral white cell count. Even with very severe peripheral leucopenia, full treatment should be continued if the marrow remains actively cellular with predominantly primitive cells.

When the initial clinical state of patients with lymphoblastic leukaemia is less severe and not deteriorating rapidly, control with antimetabolites alone may be attempted from the outset, steroids being reserved for later emergencies. The same sequence of partial aplasia followed by normal regenerative activity will then develop in responsive patients after a variable period, usually between 1 and 4 weeks. Apart from the development of marrow aplasia, a second indication for temporary cessation of treatment is the appearance of marked antimetabolite effects on the gastro-intestinal tract, but in each case maintenance therapy in reduced dosage can usually be recommenced after a short interval.

In myeloblastic, acute monocytic or undifferentiated stem-cell leukaemias the general plan of initial treatment with antimetabolites in less acutely ill patients may be followed in the same way as for lymphoblastic cases, but when the clinical state is poor at the outset, differences of opinion exist as to the desirability of giving steroid hormones. Prednisone may occasionally exacerbate the deterioration in myeloblastic leukaemia and is rarely effective except in massive doses in monocytic leukaemia, but nevertheless a trial of prednisone in combination with an appropriate antimetabolite is probably worth while in every acutely ill patient, with the proviso that continued aggravation of the clinical or haematological picture after the first few days of treatment should lead to withdrawal of the hormone.

In all cases of acute leukaemia in children, folic acid antagonists are a reasonable first choice of antimetabolite, since they are variably effective in all forms of the disease and probably give rise to the most complete and longest remissions. When a resistant state emerges or the dose required to maintain control becomes so high that toxic side-effects are intolerable, a change to 6-mercaptopurine may be effected, prednisone being used to

60 or 70 per cent of cases are resistant to treatment, and even though the leucocyte count can usually be brought down by aggressive antimetabolite therapy, no regenerative haemopoietic response occurs and primitive cells increase rapidly once more when the antimetabolite dosage is reduced below toxic levels. The existence of such a large percentage of resistant cases tends to obscure the real benefits of treatment in the smaller number of sensitive cases when group statistics are considered. Subdivision of patients with acute leukaemia into responsive and unresponsive groups, as reported by Frei and his associates (1958), shows that those of whatever age-group who respond to therapy survive almost three times as long as those who fail to respond. The failure of specific therapy to improve considerably the overall survival in adult acute leukaemia should not, therefore, be allowed to obscure the substantial improvements obtained in a minority of patients. In attempting prognosis of individual cases the initial response to therapy is most important. When the leukaemic cells appear sensitive and evidence of remission develops, the likely survival period may be considered in terms of 10 to 14 months, whereas failure to respond initially suggests that the patient is unlikely to live more than 4 to 6 months. These figures apply to both children and adults, but, of course, the majority of children with acute leukaemia will fall into the first group, while the majority of adults will fall into the second.

The periods of survival reported in the literature have usually been measured from the time of onset of the first conspicuous leukaemic symptoms, but there is obviously considerable possibility of error in assessing when the disease began, particularly in older patients who commonly manifest an insidious onset. This factor undoubtedly contributes to the rather wide scatter of recorded survival times, but it does not invalidate the general conclusions on the effect of therapy.

Illustrative Case Reports

CASE No. 1 (Fig. 37). *Acute myeloblastic leukaemia.* Male, aged 38 years, was first seen on December 30th, 1953, when he complained of increasing weakness, breathlessness and pallor for the previous 3 weeks. He had also throbbing headaches, feverishness and sweating, and anorexia. He was clinically grossly anaemic and had a few purpuric spots on the skin and buccal mucosa and several fundal haemorrhages. There were no palpably enlarged lymph glands, and neither liver nor spleen could be felt. The only physical sign specifically suggesting acute leukaemia rather than aplastic anaemia was conspicuous sternal tenderness. A blood count showed haemoglobin 2·3 gm. per cent, platelets 50,000 per cu. mm. and leucocyte count 5,200 per cu. mm., with 58 per cent myeloblasts and 4 per cent promyelocytes. Sternal marrow was very cellular and characteristic of acute myeloblastic leukaemia, with 67 per cent myeloblasts and 11 per cent promyelocytes. Despite repeated transfusions and treatment with cortisone, 200 mgm. daily, and, later, 6-mercaptopurine, 200 mgm. daily, no significant remission could be induced, the peripheral leucocyte count remained virtually unchanged, and after 7 weeks he died with a terminal broncho-pneumonia which failed to respond to antibiotics. The total course from onset was about 10 weeks.

Noteworthy features of this case include the rapid onset, the absence of glandular enlargement and of swelling and ulceration of the buccal mucosa, the presence of very severe anaemia and of thrombocytopenia with a subleukaemic leucocyte picture, the

the use of antimetabolites and hormones. Whereas, of 218 untreated cases collected from the literature by Tivey (1952), 50 per cent survived 3·9 months from the onset of their disease, 50 per cent of 154 children treated at the Memorial Hospital with folic acid antagonists and steroids survived 8·9 months from onset, and 50 per cent of 100 children treated after mercaptopurine also became available survived between 11 and 12 months. These are among the more optimistic figures published. At the other extreme, Scott (1957) found the mean length of survival from first onset of symptoms to be 20·2 weeks for 81 patients, both adults and children, treated by transfusion and antibiotics alone, while the mean survival was increased only to 21·7 weeks in 63 patients receiving also "specific" therapy with steroids or antimetabolites. Haut and his associates (1955) compared the longevity of 78 patients (45 children and 33 adults) with acute leukaemia treated with all available agents between 1947 and 1954 with the longevity of a group of 577 previously reported cases treated without specific antimetabolites or hormones. This study failed to show a statistically significant increase in survival of the specifically treated patients, either considered as a whole, or separated into childhood and adult groups. In a later report from the same centre (Wintrobe *et al.*, 1958) these earlier figures were compared with the survival data for 89 cases of acute leukaemia treated between 1954 and 1957, and an increase in median survival had now been found. The summarized data were as follows. The control group, treated before specific agents came into use, had a mean longevity of 4 months. The mean survival of patients in the 1947–54 group was 5½ months for children and 5 months for adults, and in the 1954–7 group these figures had risen respectively to 10 months and 6½ months. It is clear that the increase in longevity applied particularly to children, but this was probably a reflection of the higher incidence of lymphoblastic leukaemia in childhood, since a similar increase in longevity (from 6½ to 10 months) was found in lymphoblastic leukaemias considered as a whole, irrespective of age. In myeloblastic leukaemia the increase in mean survival (from 4½ to 5½ months) between the 1947–54 and the 1954–7 groups was not statistically significant.

While comparisons of this sort suggest that an increase in longevity of some few months is the most that has been achieved by the advances in treatment of the past 10 years, they are in a sense misleading, and are not very helpful in determining prognosis in individual patients. This is because the benefits of antimetabolite and hormone therapy are found in only a proportion of treated patients, while the considerable remainder, greater in adults than in children, fail entirely or almost entirely to respond. The position is somewhat analogous to the response of staphylococcal infections to penicillin; a proportion, due to penicillin-sensitive organisms, respond well, while the remainder, caused by resistant organisms, respond poorly or not at all. It would clearly be misleading so far as the prognosis of an individual case of staphylococcal infection is concerned to think in terms of the average response irrespective of whether the causative organism is penicillin resistant or sensitive; the differences between these subdivisions are too great. In the same way, cases of acute leukaemia may be subdivided into those resistant to therapy and those showing a good or partial response. The longevity of resistant cases is substantially unaffected by specific therapy, while that of sensitive cases is considerably increased. In lymphoblastic leukaemia of childhood nearly all cases are sensitive to one or other of the available agents and often to more than one; the improvement in survival is therefore obvious even in group surveys. In other forms of leukaemia, especially in adults, as high a proportion as

daily, and considerable temporary improvement ensued, but after 5 weeks fluid retention became severe, with peripheral and pulmonary oedema, and the cortisone was tailed off. Immediate relapse again took place, but a third remission, more substantial than the second and lasting 3 months, was induced by a *combination of 6-mercaptopurine and* cortisone. The final relapse, with death from cerebral haemorrhage, took place 16 months after the first recognized onset.

This case illustrates the resemblance to rheumatic fever sometimes shown in the initial stages of acute leukaemia, the generalized lymphadenopathy and splenomegaly most often

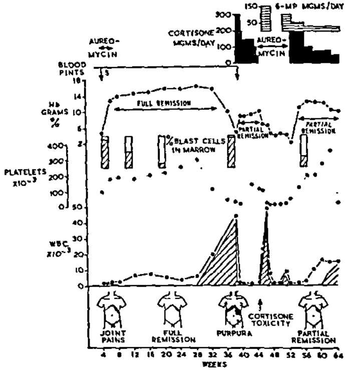


FIG. 38.

seen in the lymphoblastic form, an unusually full remission following treatment with blood transfusion and antibiotics alone, and two subsequent shorter remissions induced respectively by cortisone and combined cortisone and 6-mercaptopurine.

CASE NO. 3 (Fig. 39). *Acute monocytic leukaemia.* Female, aged 43 years, was completely well until mid-November 1954, when she developed a sore throat and fever of 104°F. She was treated with penicillin lozenges and sulphonamides and gradually improved, but 2 weeks later her gums began to swell rapidly, without soreness or bleeding, and she became suddenly very much weaker and scarcely able to stand. During the next week a petechial rash appeared on the legs and abdomen, the gum-swelling increased until the

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CASE No. 2 (Fig. 38). *Acute lymphoblastic leukaemia.* Male, aged 16 years, first noticed loss of appetite and increasing malaise in May 1953. A month later his left ankle b... swollen, hot, stiff and painful, and soon afterwards the right ankle became s... affected. He was admitted to hospital and found to have anaemia, leucopenia and throm bocytopenia, with fever, painful swollen ankles, and moderate splenomegaly and lymph-adenopathy. There were primitive cells in the peripheral blood, and a sternal marrow,

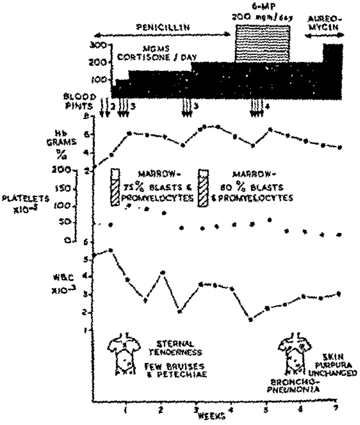

FIG. 37.

though poorly cellular, showed 90 per cent lymphoblasts. He was treated with whole blood transfusion, a total of 6 pints being given, and also antibiotics. A very full haematological and clinical remission ensued, during which both marrow and peripheral blood showed normal cytology and the only abnormal physical sign was just-detectable splenomegaly. After 6 months' remission severe relapse rapidly developed, with gross splenomegaly, moderate lymphadenopathy at all palpable sites, and subconjunctival and buccal haemorrhages. The haemoglobin fell to 4 gm. per cent, and the platelets to 10,000 per cu. mm., while the leucocyte count rose to 50,000 per cu. mm., mostly lymphoblasts. Cortisone was given in a dosage of 300 mgm. daily, later reduced gradually to 100 mgm.

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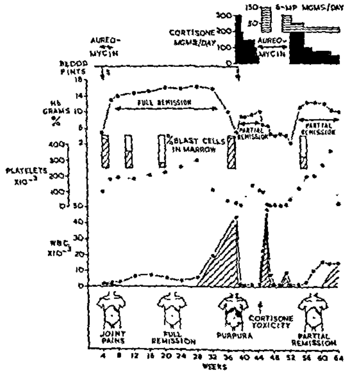

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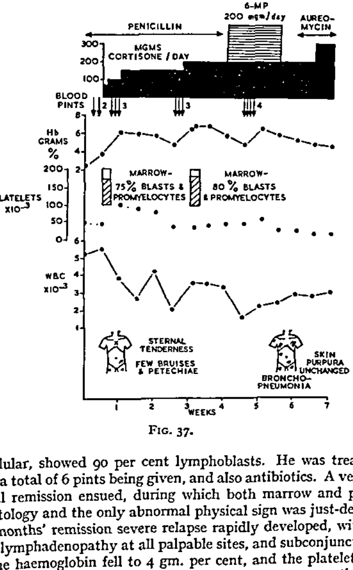

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nausea and vomiting. A course of demecolcine, 3 mgm. daily, was therefore given for a period of 7 weeks, but although the leucocyte count was again brought down to less than 10,000 per cu. mm., the haemoglobin continued to fall rapidly and several transfusions were required. Clinical deterioration slowly progressed and death occurred at the end of August, 9 months after the date of onset.

This case showed several typical features of acute monocytic leukaemia, including the gross hypertrophy of the gums, the presence of only slight enlargement of lymph glands and spleen, and the generally refractory nature of the disease. A partial remission was

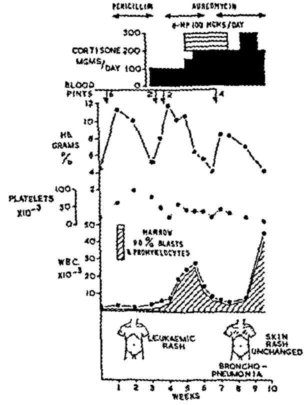

FIG. 40.

induced by 6-mercaptopurine, but demecolcine succeeded only in reducing the leucocyte count without otherwise affecting the disease state.

CASE NO. 4 (Fig. 40). *Acute myeloblastic leukaemia*. Female, aged 44 years, first noticed "rheumatic pains" in the shoulders, worse on movement, in October 1954. The pains improved after a few days, but she began to feel tired and weak and unable to do housework. After a month she developed a macular skin rash, starting on the abdomen, spreading all over the body, and becoming confluent in 2 days. There were some associated

teeth were half-submerged, and headache, great lassitude, and dyspnoea on slight exertion developed. Physical examination at this stage revealed extreme hyperplasia of the gum tissues (Plate XVII) accompanied by swelling and injection of the fauces and a few buccal haemorrhages. An extensive purpuric rash covered the abdomen and thighs. There were a few small glands palpable in the neck and axillae, and both spleen and liver were slightly enlarged. A blood count showed haemoglobin 9·6 gm. per cent, platelets 90,000 per cu. mm. and leucocytes 100,000 per cu. mm., with 92 per cent promonocytes. Treatment was started with 6-mercaptopurine, 150 mgm. daily, but although the leucocyte count fell progressively to below 10,000 per cu. mm. during the next 4 weeks, there was no reticulo-

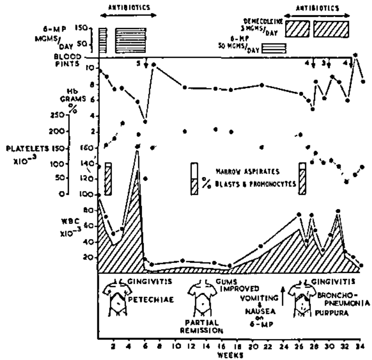

FIG. 39.

cytosis or sustained rise in the platelet level and the haemoglobin continued to fall. A respiratory infection was treated with penicillin and aureomycin, and a blood transfusion of 5 pints was given. After 6 weeks' treatment signs of remission appeared, with disappearance of purpura and fever and rise in platelets to 150,000 per cu. mm. The haemoglobin, which had been raised from a minimum of 3·2 gm. per cent to 10·3 gm. per cent by transfusion, fell more slowly to 7·5 gm. per cent and remained steady at about this level for the next 4 months, while the leucocyte count also persisted at about 10,000 per cu. mm., with 20 to 40 per cent of promonocytes. Maintenance with 6-mercaptopurine was not given during this time. In April 1955 the leucocyte count began to rise once more and the haemoglobin and platelets to fall, and 3 weeks' treatment with 6-mercaptopurine failed to affect the process of relapse, and the drug had to be discontinued because of

stantial reduction in the mediastinal gland mass. The leucocyte count fell rapidly to 46,000 per cu. mm., but soon rose again, and a week after completion of the course of deep X-rays was 87,000 per cu. mm. The clinical condition was now reasonably satisfactory, however, and the patient was allowed to go home on oral treatment with 200 mgm. of cortisone daily. The leucocyte count continued to rise rapidly despite this treatment, and the haemoglobin level now began to fall, and after 3 weeks on cortisone the figures were: haemoglobin 8·5 gm. per cent, platelets 100,000 per cu. mm. and leucocyte count 343,000

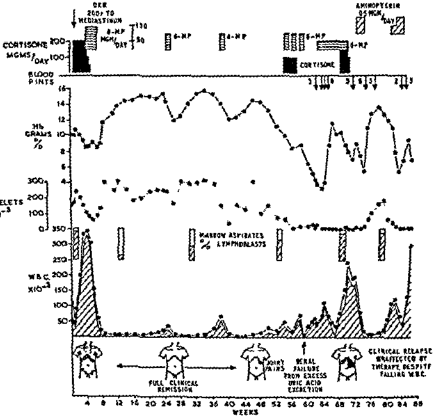

FIG. 41.

per cu. mm., predominantly lymphoblasts. *He was readmitted to hospital on December* 1st, the cortisone was stopped, and treatment with 6-mercaptopurine was started in a dosage of 150 mgm. daily, continued for 17 days. During this period the leucocyte count fell gradually to 59,000 per cu. mm., while the haemoglobin and platelets were well maintained and the clinical state improved. A very full clinical and haematological remission ensued, lasting 4 months, without maintenance therapy, and subsequent relapses were treated with short courses of 6-mercaptopurine, each producing remission, until March 1956, when a refractory state developed. The policy of intermittent short courses of

petechiae but no frank bleeding from mucosae or elsewhere. She felt feverish and sweated freely, and at this time became increasingly deaf and began to lose visual acuity in both eyes. On physical examination in early December the skin rash was seen to be a generalized rose-coloured maculo-papular eruption, confluent on the body and involving the face and limbs (Plate XVII). There were haemorrhages and conspicuous patches of exudate in both fundi, with general oedema of the retinae. A few small petechiae were present on the palate, but the mouth was not otherwise affected. There were no palpable glands, neither the liver nor the spleen could be felt, and there was no evidence of involvement in other systems. A blood count revealed haemoglobin 4·5 gm. per cent, platelets 20,000 per cu. mm., and leucocytes 2,100 per cu. mm. with no primitive cells. Sternal marrow examination showed some 90 per cent of the marrow cells to be myeloblasts or promyelocytes, thus establishing the diagnosis of acute myeloblastic leukaemia. Biopsy confirmed that the skin rash was due to infiltration by myeloblasts and promyelocytes. Treatment with cortisone, 6-mercaptopurine and blood transfusions failed to induce remission, and antibiotics did not prevent the continuance of fever, for which no causative infection could be demonstrated. Deterioration continued for 2 months, and a terminal respiratory infection led to death 4 months after the onset. The peripheral blood, initially aleukaemic, became subleukaemic, with a total leucocyte count between 2,000 and 8,000 per cu. mm. but with some 20 to 60 per cent of primitive cells, until the last few days of life, when the total count rose to 44,000 per cu. mm., with 99 per cent myeloblasts.

This case-history illustrates a prodromal rheumatic picture, with the first prominent feature a spreading leukaemic skin rash, followed by failing sight and hearing. The failure of specific therapy to influence the course of the disease is unfortunately typical of many myeloblastic leukaemias, especially in adults. The absence of buccal ulceration or gum infiltration, despite the extensive skin involvement, is noteworthy as a point of clinical distinction between myeloblastic and monocytic leukaemia, while the absence of palpable enlargement of spleen, liver or lymph glands also suggests the myeloblastic rather than the lymphoblastic or monocytic form. These clinical points of differentiation are suggestive rather than diagnostic, but in this case are supported by the clear-cut haematological findings.

CASE NO. 5 (Fig. 41). *Acute lymphoblastic leukaemia.* Male, aged 33 years, was admitted to hospital in October 1954 in a stuporose condition, with acute superior mediastinal obstruction due to a huge mass of enlarged mediastinal glands. There was generalized lymphadenopathy, the liver and spleen were both moderately enlarged, and the peripheral leucocyte count was 110,000 per cu. mm., with 83 per cent lymphoblasts. The haemoglobin level was 10 gm. per cent and the platelets numbered 150,000 per cu. mm. Treatment with oxygen relieved the dyspnoea and he regained consciousness after 4 hours. His first symptoms had apparently been dyspnoea on exertion and a feeling of respiratory obstruction, present for 2 to 3 months before admission. He had also noticed recent loss of weight and intermittent swelling of face and neck. The diagnosis of acute lymphoblastic leukaemia was confirmed by examination of the sternal marrow, and in view of the emergency state due to the large mass of mediastinal glands, X-ray therapy was given to the superior mediastinum and trachea, a total central dose of 200 r being given in three treatments over a period of 5 days. The respiratory obstruction and the congestion of face and neck were rapidly relieved and a further radiograph of the chest showed sub-

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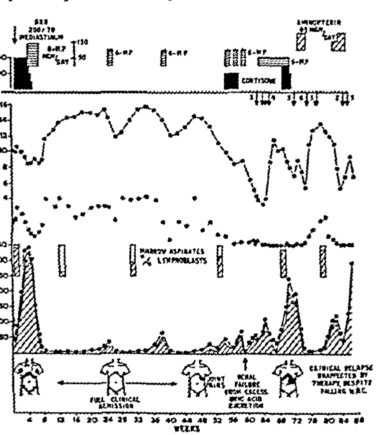

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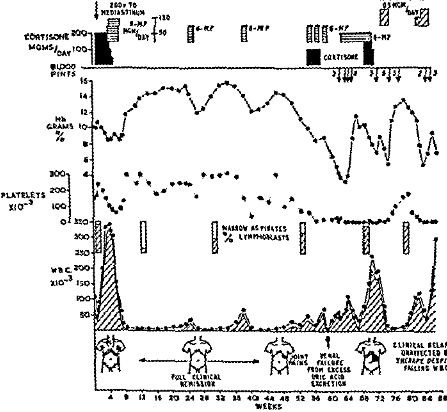

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This case-history illustrates a prodromal rheumatic picture, with the first prominent feature a spreading leukaemic skin rash, followed by failing sight and hearing. The failure of specific therapy to influence the course of the disease is unfortunately typical of many myeloblastic leukaemias, especially in adults. The absence of buccal ulceration or gum infiltration, despite the extensive skin involvement, is noteworthy as a point of clinical distinction between myeloblastic and monocytic leukaemia, while the absence of palpable enlargement of spleen, liver or lymph glands also suggests the myeloblastic rather than the lymphoblastic or monocytic form. These clinical points of differentiation are suggestive rather than diagnostic, but in this case are supported by the clear-cut haematological findings.

CASE NO. 5 (Fig. 41). *Acute lymphoblastic leukaemia.* Male, aged 33 years, was admitted to hospital in October 1954 in a stuporose condition, with acute superior mediastinal obstruction due to a huge mass of enlarged mediastinal glands. There was generalized lymphadenopathy, the liver and spleen were both moderately enlarged, and the peripheral leucocyte count was 110,000 per cu. mm., with 83 per cent lymphoblasts. The haemoglobin level was 10 gm. per cent and the platelets numbered 150,000 per cu. mm. Treatment with oxygen relieved the dyspnoea and he regained consciousness after 4 hours. His first symptoms had apparently been dyspnoea on exertion and a feeling of respiratory obstruction, present for 2 to 3 months before admission. He had also noticed recent loss of weight and intermittent swelling of face and neck. The diagnosis of acute lymphoblastic leukaemia was confirmed by examination of the sternal marrow, and in view of the emergency state due to the large mass of mediastinal glands, X-ray therapy was given to the superior mediastinum and trachea, a total central dose of 200 r being given in three treatments over a period of 5 days. The respiratory obstruction and the congestion of face and neck were rapidly relieved and a further radiograph of the chest showed sub-

stantial reduction in the mediastinal gland mass. The leucocyte count fell rapidly to 46,000 per cu. mm., but soon rose again, and a week after completion of the course of deep X-rays was 87,000 per cu. mm. The clinical condition was now reasonably satisfactory, however, and the patient was allowed to go home on oral treatment with 200 mgm. of cortisone daily. The leucocyte count continued to rise rapidly despite this treatment, and the haemoglobin level now began to fall, and after 3 weeks on cortisone the figures were: haemoglobin 8·5 gm. per cent, platelets 100,000 per cu. mm. and leucocyte count 343,000

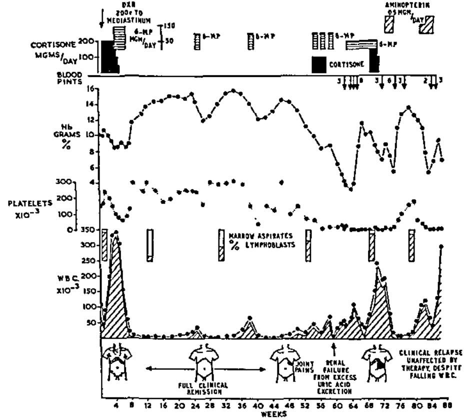

FIG. 41.

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were similarly induced. The danger of renal obstruction and uraemia following a rapid decrease in the leucocyte count is also illustrated.

CASE No. 6 (Fig. 42). *Acute lymphoblastic leukaemia.* Male, aged 3 years, was first admitted to hospital in November 1956 with a history of increasing pallor and tendency to bruise for 8 weeks. He was purpuric and febrile and had moderate hepato-splenomegaly. The peripheral-blood count showed severe anaemia, haemoglobin 5·4 gm. per cent, thrombocytopenia, platelets 100,000 per cu. mm., and an aleukaemic leucopenic white cell picture. Marrow aspiration established the diagnosis of acute lymphoblastic leukaemia, smears being composed predominantly of primitive cells. Initial treatment with transfusion and prednisone induced a full remission, which was maintained for 7 months on amethopterin before a resistant state developed. Chemotherapy was then continued with 6-mercaptopurine, prednisone and transfusions being given to assist control during the period of changeover. A second full remission ensued for 3 months before resistance again emerged. A third short and partial remission, lasting 6 weeks, followed the combined use of prednisone, amethopterin and transfusions, but the continued use of all these agents together with 6-mercaptopurine could not prolong remission further and a mixed aplastic-leukaemic terminal state, with staphylococcal laryngitis, led to death 17 months after the first onset of symptoms.

The use of transfusions, steroids and antimetabolites in sequence and in combinations is illustrated by this case report, which also provides an example of the excellent remissions often to be obtained in lymphoblastic leukaemia of childhood. The initially aleukaemic picture, with no primitive cells in the peripheral blood, made marrow aspiration essential to confirm the suspected diagnosis.

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treatment, given when the leucocyte count began to rise sharply, was successfully carried out on an outpatient basis, but on one occasion, when the leucocyte count was brought down from 72,000 to 12,000 in a short period, renal colic and uraemia developed, with blood urea 220 mgm. per cent and blood uric acid 18 mgm. per cent. and a short admission to hospital for fluid and dietary control and transfusion was necessary.

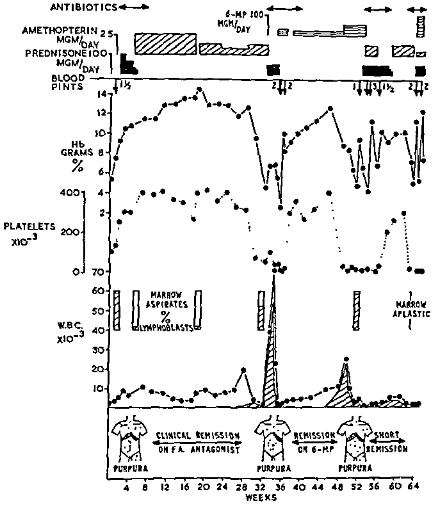

FIG. 42.

Further attempts to induce remission with aminopterin, after the disease had become resistant to 6-mercaptopurine, were unsuccessful, and death occurred in May 1956, 22 months after the first onset of symptoms.

This case illustrates a rather unusual initial clinical picture of progressive respiratory difficulty leading to acute mediastinal obstruction, relieved by local X-ray treatment. Treatment with cortisone was unsuccessful, but a full and prolonged remission was induced by a single course of 6-mercaptopurine, and subsequent shorter partial remissions

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patient and frequently gives rise to unpleasant dragging sensations or feelings of fullness in the left side of the abdomen. Sometimes there is acute pain in the splenic region, aggravated by respiratory movements and perhaps referred to the left shoulder, probably due to perisplenitis over an infarcted zone. The enlarged spleen may cause digestive disturbances, with flatulence and anorexia and even occasionally vomiting, by displacing and compressing the stomach. Metabolic symptoms are less common and conspicuous, but the raised basal metabolic rate may be associated with loss of weight, increased sweating, nervousness, fever and tolerance of cold.

Retinal or skin haemorrhages, haematuria, or bleeding from mucous membranes of the nose, gastro-intestinal tract or uterus occur early in the course of the disease in some 20 to 30 per cent of patients, but are rarely as severe as those encountered in acute leukaemia.

Among less common initial symptoms are bone pains, usually in the legs or hips but sometimes in the back. These tend to be poorly localized and are not often severe enough to be the prime symptoms. Priapism, venous thromboses, and menstrual irregularities or amenorrhoea are also occasionally prominent initially, and pressure from splenic enlargement may cause herniation or uterine prolapse, while rarely the development of intense pruritus or a skin rash may first lead the patient to consult his doctor.

Scott (1957) analysed the frequency of different presenting symptoms in 177 patients with chronic granulocytic leukaemia and found asthenia in 58·2 per cent, awareness of an abdominal tumour in 44·1 per cent, abdominal pain in 41·3 per cent, loss of weight in 40·1 per cent, symptoms of anaemia in 31·1 per cent, a haemorrhagic state in 26·1 per cent, dyspepsia in 24·9 per cent, bone pains in 9·6 per cent, ankle oedema in 5·6 per cent, and hernia in 3·9 per cent.

The physical signs found on initial clinical examination closely parallel the predominating symptoms. Some degree of anaemia is usually present, the haemoglobin level being between 6 and 10 gm. per cent in the majority of patients. Obvious loss of weight is another very common feature, but the most striking abnormality is certainly splenomegaly. The spleen is almost invariably considerably enlarged, reaches to below the umbilicus in most patients, and not infrequently fills the whole left side of the abdomen, extending far across the midline and inferiorly into the pelvis. The lymph glands are much less often enlarged, but slight or moderate generalized lymphadenopathy is met with in about 20 to 30 per cent of patients. Hepatomegaly is often demonstrable but is rarely gross. Other initial physical signs are inconstant, but among those seen from time to time are purpura and skin rashes, retinal haemorrhages, bone tenderness, most common over the sternum, and other signs of system involvements discussed more fully below.

System involvement and correlated pathology

Lymph glands. While, as we have seen, enlargement of lymph glands is not common initially in chronic granulocytic leukaemia, involvement becomes a more frequent feature during the later course of the disease and the majority of patients have some generalized lymphadenopathy before their illness has run its course. The glands remain discrete and are not tender. Histologically they show extensive infiltration of the normal glandular reticulum with granulocytes of every developmental stage, and in some cases there is little lymphocytic tissue remaining (Plate XIX). Rarely the lymph glands in one or more

CHAPTER 13

CHRONIC GRANULOCYTIC LEUKAEMIA: CLINICAL ASPECTS

THE clinical and haematological features of chronic granulocytic leukaemia are more uniform than those of acute leukaemia. Problems of differential diagnosis from non-leukaemic diseases, particularly from other members of the myeloproliferative group and from leukaemoid reactions, arise in a small proportion of cases, and are discussed in Chapter 16. Subdivisions within the chronic granulocytic class seldom cause diagnostic difficulty, and eosinophilic, basophilic and mature neutrophilic leukaemias are extremely uncommon. These rare forms of granulocytic leukaemia are briefly described in Chapter 15, and attention is confined in the present chapter to the common form of chronic granulocytic or myelocytic leukaemia, its modes of onset, initial symptoms and signs, system involvements and correlated pathological changes, general clinical differential diagnosis, treatment, course and prognosis. Case-histories illustrating the clinical aspects and the use of modern chemotherapy are appended.

The incidence of this form of leukaemia in relation to age, sex, habitat and environmental factors has already been discussed (Chapter 3) and we have seen that the disease is rare in childhood but increasingly common with advancing age, and that males and females are affected with about equal frequency.

Modes of onset

The onset is usually insidious, symptoms slowly developing over a period of months or even years before they become severe enough for the patient to seek medical advice. Minot, Buckman and Isaacs (1924) and Hoffman and Craver (1931), in analyses of several hundred case-histories, found intervals averaging a little over a year between the time that symptoms were first noticed and the time that a firm diagnosis was made. The disease may, however, be present long before any noticeable symptoms occur, and there are several reported cases in which the blood picture of chronic granulocytic leukaemia was discovered in apparently healthy individuals one or more years before the first onset of symptoms (Wintrobe and Hasenbush, 1939; Sturgis, 1955). In some of these patients physical examination revealed no abnormality, while in others there was slight enlargement of the spleen or lymph glands. The frequent finding of gross splenomegaly at first examination of patients with chronic granulocytic leukaemia itself indicates that the disease must have been developing for some considerable time in the great majority of patients.

The initial complaints are chiefly those resulting from slowly progressive anaemia, from splenomegaly, or from metabolic disturbances. Thus anaemia causes pallor, weakness, lassitude, palpitations and dyspnoea on exertion, and these symptoms are among those most commonly encountered, while splenic enlargement may itself be noticed by the

(Scott, 1957), but it occurs far more often in chronic lymphocytic leukaemia (Katz, 1932; Forkner, 1938; Wile and Holman, 1940).

Specifically leukaemic skin lesions are rare. Barney (1933) collected details of 21 reported cases, and described the commonest pattern as consisting of firm, circumscribed nodules or plaques, grey-blue or brown in colour, varying in size from 1 mm. to 5 cm. in diameter, found chiefly on the trunk and seldom seen on the face, scalp or extremities. The lesions are usually painless, but ulceration and infection may develop, especially when the nodules are large enough to cause bulging and stretching of the overlying skin, and pain may then occur. Histologically there is a thinning and flattening of the epidermis, and dense infiltration of the corium and subcutaneous tissue with granulocyte precursors. There is some evidence that the appearance of leukaemic skin nodules in chronic granulocytic leukaemia heralds rapid deterioration and that death is likely to follow within a few weeks (Goldhamer and Barney, 1936; Paul and Limarzi, 1942; Scott, 1957).

Bones and joints. The intense proliferation of leucopoietic tissue in the bone marrow in chronic granulocytic leukaemia might be expected to lead to thinning or erosion of both medullary and cortical bone, with the production of radiologically demonstrable bone lesions. Such lesions are rather surprisingly rare, however, occurring in only about 5 per cent of patients, although minor destructive foci, not visible in radiographs, may be detected in a much higher proportion of cases at autopsy (Craver and Copeland, 1935). Since the majority of patients are adults, disturbances of bone growth like those found in children with acute leukaemia are not to be expected, but localized tumours, areas of focal bone destruction leading sometimes to pathological fractures, and subperiosteal infiltration with or without new bone formation have all been observed (Silverman, 1948; Nesbitt and Roth, 1955; Scott, 1957). These advanced skeletal lesions have been found chiefly in the upper parts of the femur or humerus, in the pelvis or in the lumbar spine, but some generalized reduction in bone density is not uncommon (Plate XXV, Fig. 1). Bony tenderness on firm pressure may often be detected, especially over the body of the sternum, and Craver (1927) found this sign present in 75 per cent of patients with chronic granulocytic leukaemia, although, as he pointed out, the area of tenderness may be quite small and localized and found only after careful search.

Pain and limitation of joint movements, perhaps associated with swelling and effusion, have been reported occasionally in chronic granulocytic leukaemia, particularly in the rare cases in childhood. The joints chiefly affected have been the knees, elbows, wrists or finger joints, and post-mortem examination has revealed leukaemic infiltration of the synovial membranes and juxta-articular bony erosions (Bedwell and Dawson, 1954).

Central nervous system. Neurological lesions may arise during the course of chronic granulocytic leukaemia as a result of haemorrhage secondary to thrombocytopenia in the later phases of the disease, or as a consequence of thrombosis, regional infiltration with leukaemic cells, or local tumour formation. Examples of these complications have long been recognized and reported (Bramwell, 1886; Eichhorst, 1898; Stursberg, 1914; Schwab and Weiss, 1935) and the extensive earlier literature has been reviewed by Forkner (1938). The symptoms and signs vary according to the nature and distribution of the lesions from headache and mental disorientation to cranial or peripheral nerve palsies, hemiplegia, paraplegia, peripheral sensory abnormalities, meningeal signs and coma. Cerebral or cranial nerve involvement is more common than spinal or peripheral nerve lesions, but,

groups may be markedly enlarged and perhaps painful and tender, and this uncommon finding has been thought to presage a rapidly progressive course (Emile-Weil and Isch-Wall, 1930; Scott, 1957). The possibility that gross localized lymphadenopathy in chronic granulocytic leukaemia may be due to a second concurrent disease process, such as Hodgkin's disease or lymphosarcoma, must be borne in mind, since cases of this sort have been reported (Skworzoff, 1930; Forkner, 1938).

Spleen. The remarkable extent of splenic enlargement in this form of leukaemia is so consistent that even in treated cases, where the spleen size may have been greatly reduced during periods of satisfactory control, the average weight of the spleen at autopsy approaches 2 kilograms. The organ retains its characteristic shape, with a well-marked notch and generally smooth surface, but is very much firmer in consistency than a normal spleen. There may be areas of surface roughening and creamy-white patches of fibrous perisplenitis overlying infarcted areas, and adhesions to the abdominal wall or diaphragm are commonly found. The cut surface of the spleen is paler than normal and an impression of diffuse greyish-white infiltrate may be gained. Yellow or white infarcts, in various stages of fibrous organization, are common, and areas of haemorrhage may be seen. Histologically the capsule and trabeculae can be distinguished clearly, but the remaining pattern of splenic structure is heavily infiltrated and replaced by polymorphonuclear cells and granulocyte precursors and the Malpighian bodies can hardly be discerned.

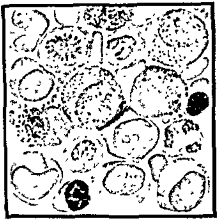
Liver. Enlargement of this organ is seldom marked in the earlier stages, but becomes more prominent as the disease progresses and at autopsy it is usual to find considerable and even gross hepatomegaly. The surface is smooth and the consistency rather soft, while on section there may be seen evidence of infiltration of a diffuse rather than a nodular kind. The colour is pale, with a greyish or yellowish tinge. Histologically the normal pattern of hepatic architecture is well preserved, but extensive infiltration with granulocytes, both mature and immature, is to be seen in the areas around the portal tracts and along the hepatic capillaries and sinusoids (Plate XXV, Figs. 6 and 7). Hepatic involvement seems rarely to interfere substantially with the functions of the liver, and jaundice or ascites due to liver damage are most uncommon.

The skin and oral mucous membranes. The commonest cutaneous lesions in chronic granulocytic leukaemia are due to haemorrhage and occur principally in the later stages of the disease when the platelet count is low. A fully purpuric picture may then develop, similar to that frequently seen in acute leukaemia, but widespread and severe purpura is seldom seen in the chronic disease except in the terminal stages or when aggressive therapy had led to too sharp a fall in the platelet count. Petechial haemorrhages of the buccal mucosa or bleeding from the gum margins may also be found in chronic granulocytic leukaemia, but although they may sometimes occur early in the disease they are usually of minor extent except in the terminal thrombocytopenic phases.

Apart from purpura, a wide variety of non-specific skin rashes have been reported in chronic leukaemias, but they mostly occur in lymphocytic leukaemia, and in the granulocytic form there is little convincing evidence that any form of cutaneous rash can justifiably be regarded as a leukaemid. Patients suffering from granulocytic leukaemia do not of course lose their general susceptibility to skin diseases, but none appears unduly common, except perhaps herpes zoster. The incidence of this disease is certainly higher than could be explained by coincidence, being found for example in 7 of Scott's 177 patients

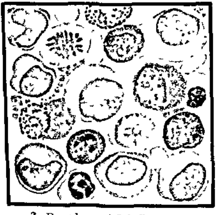
PLATE XXIV

CYTOLOGY AND CYTOCHEMISTRY OF CHRONIC GRANULOCYTIC LEUKAEMIA

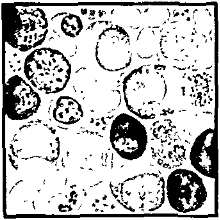


1. May–Grunwald Giemsa stain

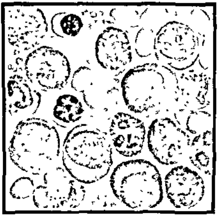
2. Feulgen reaction



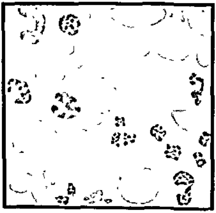
3. Periodic acid Schiff reaction



4. Sudan black B stain



5. Peroxidase reaction



6. Alkaline phosphatase reaction

apart from terminal cerebral haemorrhages, all neurological complications are quite rare. Scott (1957) did not note a single example among the 177 patients included in his survey.

Eyes and ears. Visual symptoms are infrequent in chronic granulocytic leukaemia, although severe haemorrhage into the vitreous or retina may rarely lead to sudden blindness, and less dramatic impairment of vision may follow repeated smaller haemorrhages. Nevertheless, examination of the eyes reveals significant abnormalities in about two-thirds of all patients. Goldbach (1933) found lesions in 60 per cent of 143 cases, the most prominent being retinal haemorrhages, tortuous retinal vessels, optic neuritis with blurring of the disc, and variable degrees of conjunctivitis and scleritis. Retinal changes occur early in the course of the disease, and haemorrhages are more common in the fundus oculi than in the skin. Leukaemic retinopathy, with venous engorgement, diffuse retinal oedema, haemorrhages and circular white exudates with surrounding haemorrhagic halos, was observed by Scott (1957) at the first examination in 11·4 per cent of his patients.

Haemorrhage or leukaemic infiltration in the auditory apparatus may lead to tinnitus, vertigo, Ménière's syndrome or nerve deafness, but such complications seldom occur in the chronic granulocytic form of leukaemia and few cases have been reported.

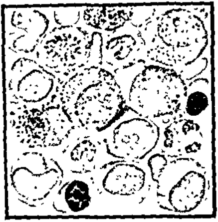
Gastro-intestinal system. Specifically leukaemic involvement of the alimentary tract is very uncommon and it is rare to find more than slight generalized infiltration with myeloid cells at autopsy. Alimentary symptoms are very often present, however, and we have already seen that dyspepsia may be a presenting feature. Anorexia, flatulence and debilitating diarrhoea may also be prominent, while gastro-intestinal haemorrhage in leukaemic patients may lead to diagnostic confusion, since the clinical picture of primary peptic ulceration may be closely simulated (Wintrobe and Mitchell, 1940). Indeed, gastric and duodenal ulcers are not uncommon in chronic granulocytic leukaemia, perhaps because the splenomegaly and increased metabolic rate throw additional strains on gastric and intestinal activity and exacerbate any existing tendency to ulcer formation. Abdominal examination in these patients will reveal considerable splenomegaly, and the blood picture will establish the existence of leukaemia. If peptic ulceration is confirmed as the source of haemorrhage by further investigations, medical treatment for the ulcer, with a gastric diet and antacids, may be combined with antileukaemic therapy. Gastro-intestinal symptoms, especially anorexia and diarrhoea, which are unassociated with a primary lesion in the gut, usually respond rapidly to any treatment which is successful in inducing remission of the leukaemic process, and call for no additional therapeutic measures.

Genito-urinary system. Haematuria is commonly observed. Locke and Minot (1924) found it in 15 per cent of 110 cases of chronic granulocytic leukaemia, and it is probably present in a minor form at some stage of the disease in the majority of patients. The bleeding may be secondary to thrombocytopenia and part of a more generalized haemorrhagic state, or it may arise as a consequence of local infiltration with leukaemic cells or tumour formation, with venous congestion and mucosal disruption.

Infiltrations may lead to enlargement of the kidneys, of the genital organs or of the prostate gland, but such involvement is rarely marked. Amenorrhoea is observed in about 10 per cent of women who develop the disease during the reproductive period of life, and menorrhagia is a frequent manifestation of thrombocytopenic bleeding. These disorders respond best to anti-leukaemic therapy, although some forms of therapy, such as busulphan, themselves induce amenorrhoea in a proportion of patients. Priapism, due to

PLATE XXIV

CYTOLOGY AND CYTOCHEMISTRY OF CHRONIC GRANULOCYTIC LEUKAEMIA



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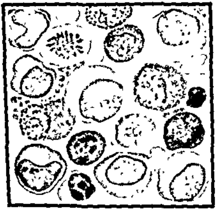
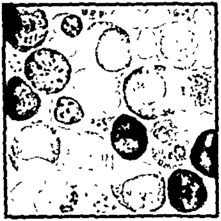


3. Periodic acid-Schiff reaction



4. Sudan black B stain

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To face p. 246

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thrombosis or leucocyte aggregation in the corpora cavernosa, is a well-recognized symptom of chronic granulocytic leukaemia in the male, and may be persistant and painful. It is much less common than many text-book descriptions would lead one to believe; probably not more than 2 to 5 per cent of males suffer from it (Craver, 1933; Scott, 1957). Priapism of the clitoris has been reported but is excessively rare. The symptom responds rapidly to general anti-leukaemic therapy, and surgical evacuation of the corpora cavernosa is not indicated.

Respiratory system. Since lymphadenopathy is seldom gross in granulocytic leukaemia, respiratory symptoms secondary to bronchial obstruction by enlarged mediastinal glands are not often encountered, but some degree of basal pulmonary congestion or collapse often results from diaphragmatic elevation due to the enlarged spleen and liver. Pulmonary infections tend to develop terminally and probably occur more readily in the presence of vascular engorgement with granulocyte precursors and consequent alveolar oedema (Plate XXV, Figs. 4 and 5). Infiltration of the pleura with the formation of an exudate is rare.

Cardiovascular system. Specific infiltration of the heart or pericardium is seldom extensive enough to give rise to symptoms or physical signs, although histological sections often disclose some invasion of the myocardium. Pericarditis and pericardial effusions have occasionally been reported (Bierman, Perkins and Ortega, 1952; Scott, 1957).

Palpitations, dyspnoea, systolic murmurs and later cardiac failure may occur as a result of anaemia.

The blood. The peripheral blood picture in chronic granulocytic leukaemia is nearly always quite characteristic and unequivocally diagnostic. The number of circulating leucocytes is enormous, averaging between 200,000 and 300,000 per cu. mm., with occasional levels greater than a million per cu. mm. Counts below 50,000 per cu. mm. at the time of first examination, prior to the start of therapy, are uncommon, and subleukaemic or aleukaemic forms are extremely rare. Differential leucocyte counts show that the great majority of the circulating cells are granulocytes or granulocyte precursors, neutrophil polymorphs, metamyelocytes and myelocytes predominating, but with variable numbers of promyelocytes and myeloblasts, usually less than 10 per cent of the total. A further conspicuous feature is an increase in basophils, which commonly make up from 5 to 20 per cent of the total, and there may also be an increase in total numbers of eosinophils, roughly in proportion to the rise in the neutrophil figures. Variability in size and granularity is often prominent among both the mature and immature granulocytes, and

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PLATE XXV

CHRONIC GRANULOCYTIC LEUKAEMIA

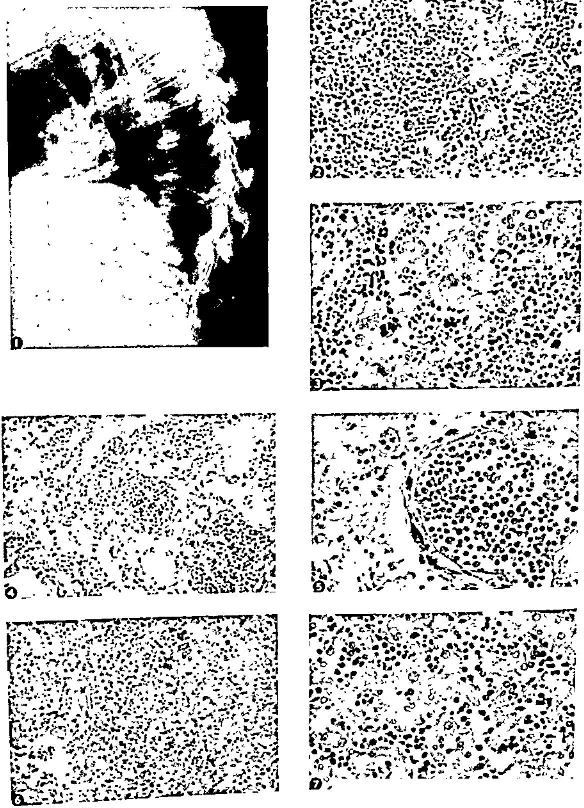
Radiological and Histological Features

1. Vertebral collapse and diffuse porotic changes.
2. Marrow section from chronic granulocytic leukaemia showing megakaryocytic hyperplasia and resembling 3. (× 100)
3. Marrow in megakaryocytic myelosis and myeloid metaplasia. (× 100)

4 and 5. Low- and high-power views showing pulmonary infiltration with immature leukaemic granulocytes. (× 50 and × 100)

6 and 7. Low- and high-power views showing characteristic pattern of hepatic infiltration in chronic granulocytic leukaemia. (× 50 and × 100)

PLATE XXV



Differential diagnosis from non-leukaemic states

The highly characteristic initial clinical picture of the great majority of patients with chronic granulocytic leukaemia makes for easy and rapid diagnosis, and even when the symptoms and signs are not typical the blood findings are almost invariably diagnostic. Clinically, confusion might exist between chronic granulocytic leukaemia and the many other causes of anaemia and splenomegaly, but there is little purpose in analysing the points of similarity and difference that might be helpful in establishing the correct diagnosis clinically, since a blood count would certainly be required in any of these conditions and would at once provide the answer in almost every case. More substantial difficulty may arise when the peripheral-blood picture is aleukaemic or leuco-erythroblastic, with only a moderate increase in leucocytes to the region of 15,000 to 50,000 per cu. mm., and in these circumstances examination of the bone marrow and cytochemical studies of leucocyte alkaline phosphatase may be necessary to distinguish between leukaemia and other myeloproliferative diseases such as myelofibrosis, and myelocytic leukaemoid reactions to acute or chronic infections, or to metastasizing malignancies and lymphomas. When an actively cellular granulocytic marrow picture is found, and the alkaline phosphatase content of the leucocytes is below normal, a diagnosis of leukaemia can be made with confidence (see also p. 313).

Treatment

The general principles of radiotherapy and chemotherapy of the leukaemias, and the range of available agents, their modes of action, toxic effects, dosages and methods of use have already been dealt with in Chapters 9 and 10, while supportive modes of treatment have been discussed in Chapter 11. Opinions on the practical use of the most appropriate agents in chronic granulocytic leukaemia will now be summarized.

Radiotherapy has been very widely used in the treatment of this disorder for more than 50 years and is highly effective in alleviating symptoms and increasing efficiency and well-being in nearly every case. The treatment is most commonly applied to the enlarged spleen, successive exposures being given through different skin areas, anterior, lateral and posterior, overlying first the lower part of the spleen and later the upper part. Satisfactory responses with minimum skin effects can be achieved in this way. The initial dose, usually of 50 to 100 r, is followed by successive daily doses of the same or gradually increasing amount in accordance with the changes in peripheral leucocyte count, so that a steady fall is brought about. Treatment is stopped when the leucocyte level has reached about 20,000 per cu. mm., and a continued fall to the region of 10,000 per cu. mm. is likely to take place during the next few days. There is a good deal of variation in the sensitivity of individual patients to irradiation, and doses ranging between 200 r and 1,200 r may be necessary to achieve the desired result. Following the drop in leucocyte count, the haemoglobin and red cell levels rise towards normal, the spleen is reduced in size and clinical remission ensues. The blood picture is followed closely after the course of deep X-ray until it becomes relatively stable and blood counts are then repeated monthly. Further courses of treatment may be given when the leucocyte count rises above 50,000 per cu. mm. or when splenomegaly or troublesome symptoms recur. Second and later courses of treatment generally involve higher doses before an adequate effect can be achieved, and

mitotic figures, sometimes irregular, may be seen in circulating myeloblasts, promyelocytes and myelocytes. Although lymphocytes and monocytes are relatively few in numbers, their absolute levels are commonly much increased above normal, and in some cases a substantial monocytosis may exist. There is no doubt that the close relationship between monocytic and myeloblastic proliferation, manifest in the acute myelo-monocytic leukaemias, has a parallel in chronic granulocytic leukaemia, since occasionally monocytes may be as numerous as myelocytes in the peripheral blood in this disease. The cytological features of the leucocytes in the blood are illustrated in Plate XXIV. Distinctively leukaemic characteristics, enabling the differentiation of individual cells from their counterparts in non-leukaemic leucocytic proliferation or leukaemoid states, cannot be detected, but there is a remarkable absence of alkaline phosphatase activity in the polymorphs in this form of leukaemia, whereas the leucocyte content of this enzyme is higher than normal in most leukaemoid states.

The platelets are usually numerous in the early stages and platelet counts much above normal are not uncommon. In some cases the platelets are markedly irregular in size and shape, and megakaryocyte fragments may be seen in the circulating blood. Subnormal platelet levels are often found in the later stages of the disease, when thrombocytopenic bleeding may develop, and this decrease in platelets appears to form part of the natural course of the leukaemia, although it is not always easy to be certain that thrombocytopenia developing during the course of treatment with radiation or cytotoxic drugs has not been therapeutically induced.

Anaemia is invariably present at some stage of the disease and is often the dominant factor in the clinical picture. Occasionally when chronic granulocytic leukaemia is first diagnosed, there may actually be a degree of polycythaemia, and the relation between these two myeloproliferative diseases will be discussed in more detail in Chapter 16. In the great majority of cases, however, moderate anaemia is present from the time of first diagnosis, usually with normocytic, normochromic red cells, and tends to become more severe as the leukaemic process deteriorates. With haemoglobin levels below 6 or 7 gm. per cent the red cells often show anisocytosis and less often poikilocytosis, and an increase in polychromasia and reticulocytes to 5 or 10 per cent may be found, while a few late normoblasts may appear in the peripheral blood. The nature of the anaemia and possible factors active in its pathogenesis have been discussed earlier (pp. 197–200).

The bone marrow. As in the acute leukaemias, actively leucopoietic greyish-red marrow is found throughout the whole skeleton, replacing the normal red marrow of the axial skeleton and the inactive fatty marrow of the limb bones. Trabecular erosions are often to be found, and there may be evident thinning of the cortex with an expanded marrow cavity. The cytology of the marrow cells in smears and sections closely resembles that of the peripheral-blood cells. Granulocytes in all stages of development are present in large numbers, with neutrophil myelocytes, metamyelocytes and polymorphs predominating. Islands of erythroblasts can be found, and although the myeloid:erythroid ratio is increased very considerably over normal, total erythropoietic activity is probably at least as great and perhaps greater than in the normal marrow. Megakaryocytes are conspicuous in the early stages of the disease when the peripheral platelet count is high (Plate XXV, Figs. 2 and 3), but when thrombocytopenia develops, megakaryocytic aplasia is usually found.

discussed the incidence and prevention of toxic reactions, especially myeloid aplasia. Galton and Till (1955), recording the results of treatment of 31 cases during a 5-year period, recalled that their initial short intensive dosage scheme, in which 100–150 mgm. were given in 1 to 6 days, was stopped because three patients developed severe marrow depression and one died. Subsequently a more conservative plan of therapy was used, with doses rarely exceeding 4 mgm. daily. Treatment was stopped "when clinical and haematological improvement seemed to justify it, or when the leucocyte count was thought to be falling too steeply". In general they have aimed to maintain the leucocyte count between 10,000 and 20,000 per cu. mm., and no further cases showing signs of toxicity were encountered.

Greig (1956) used a similar initial dosage of 4 mgm. daily, but continued treatment at this level until "the blood count showed no further evidence of improvement". The leucocyte count was brought to "normal levels", and maintenance therapy continued at a dose between 1 and 2 mgm. daily. Marrow aplasia occurred in only 1 of 34 cases in his series, and that in a patient who had inadvertently received a double dosage rate for 45 days (8 mgm. daily). Recovery took place when busulphan therapy was stopped. Greig noted that in two patients who were thrombocytopenic at the start of treatment the effect of busulphan was to aggravate this feature, and the drug had to be stopped.

A more precise plan of therapy which appears equally safe and allows more rapid remission to be produced has been used by Hyman and Gellhorn (1956). They gave initial doses of 10 mgm. daily and followed the peripheral leucocyte count every 2 to 3 days until it reached 25 per cent of the original level. Therapy was then discontinued until the leucocyte fall stopped. Further treatment was given if necessary to bring the white cell level to within the normal range of 6,000–10,000 per cu. mm. If initial response was unsatisfactory, doses of as much as 20 mgm. daily were given until a suitable rate of fall was achieved. An even more aggressive routine plan of therapy has been used by Dameshek (1957), who gave initial doses of 8–16 mgm. daily until the leucocyte count reached 5,000–6,000 per cu. mm. The drug was then stopped, and while the white cell count sometimes continued to fall to 2,000–3,000 cells per cu. mm., Dameshek encountered no deleterious effects from this procedure in a series of 34 cases, apart from 2 temporary cases of thrombocytopenia. Maintenance with doses of the order of 2–4 mgm. daily may be well tolerated and lead to prolonged remission, but unexpected bone-marrow failure has developed suddenly in a few patients so treated (Haut *et al.*, 1955; Storti and Pederzini, 1955; Hayhoe and Kok, 1957).

Clearly, the administration of large doses of busulphan is frequently associated with the development of aplastic states (Wilkinson, 1953; Galton and Till, 1955; and Storti and Pederzini, 1955). In the majority of cases high dosage rates are now used only in patients who have proved resistant to more conservative schemes of treatment, and then only with very close haematological supervision. The dosage employed by Dameshek appears to be too close to the toxicity level to be safely used without very frequent blood counts, and, although in his experience undue marrow depression has not occurred, the advantages of such intensive therapy for routine use would seem to be outweighed by the risks.

It is theoretically desirable to maintain the white cell count between 5,000 and 10,000 cells per cu. mm., and Hyman and Gellhorn emphasize that in their experience the best results of treatment with busulphan are obtained when the leucocyte level has been

the resulting remissions last for successively decreasing periods, so that it may become more satisfactory in the course of time to discontinue intermittent intensive courses of treatment and replace them by a single weekly splenic exposure of 100 to 200 r. A refractory state eventually emerges, and at this stage further control can sometimes be effected by chemotherapy.

Spray irradiation may be given to the long bones (Leucutia, 1934) and attempts have been made to calculate a maintenance dose of whole body radiation to be given at regular intervals, instead of the more customary intermittent courses (Osgood, 1951). It is doubtful if these alternative methods of treatment offer any advantage over simple splenic irradiation.

Irradiation sickness, with nausea, vomiting and diarrhoea, may be troublesome during the period of treatment, but is not often severe and is of short duration. A rare complication of treatment, when the leucocyte count falls very rapidly and the blood uric acid level rises sharply, is ureteric obstruction by uric acid crystals. Anuria and renal failure may develop (Lear and Oppenheimer, 1950). The hyperuricaemia has been known to precipitate acute gout (Shorvon, 1946). In the great majority of patients, however, splenic irradiation is well tolerated and effective. The first remission induced by this means usually lasts about 9 months to a year, but occasional patients may be found to be much more sensitive or resistant than the average and a second course of radiation may not be needed for 4 or 5 years or may be required within 3 months of the first. Treatments may be given as often as necessary to hold down the leucocyte count and the spleen size, provided that they do not aggravate anaemia or thrombocytopenia, and that signs of acute myeloblastic crisis are not present.

Radioactive phosphorus has been widely used in chronic granulocytic leukaemia, and yields remissions comparable with those obtained by X-rays (Osgood, Seaman and Tivey, 1955). The variation in individual sensitivity is considerable, and dosage must be adjusted carefully during the initial course in accordance with the changes in the blood count. The only advantages of P^{32} treatment over X-radiation are the freedom from irradiation sickness and the fact that elaborate apparatus is not required. Since chemotherapeutic agents offer these advantages and others, radioactive phosphorus is decreasingly used in the treatment of this disease at present. Recommendations for the practical management of P^{32} therapy have been given by Reinhard and his associates (1946), Lawrence *et al.* (1948), Friedell and Storaasli (1949) and Osgood and Seaman (1952).

Chemotherapeutic agents effective in chronic granulocytic leukaemia include urethane, the nitrogen mustards, triethylene melamine, busulphan (myleran), 6-mercaptopurine and desacetylmethylcolchicine (demecolcine). The undesirable side-effects associated with the administration of the nitrogen mustards and to a lesser extent urethane and triethylenemelamine, and the marked variation in sensitivity of marrow tissue in different patients, with the consequent unpredictability of response and danger of drug-induced aplasia, have resulted in the relegation of these agents from general use. Of the remaining drugs, the most effective, safest and least troublesome appears to be busulphan (see also p. 177). When used in the treatment of chronic granulocytic leukaemia, busulphan achieves excellent control for long periods in most cases. Several different schemes of treatment with a wide range of dosage have been reported, and these have been reviewed and recommendations for routine use of busulphan given by Hayhoe and Kok (1957), who also

Reference has been made earlier to the use of 6-mercaptopurine and of desacetylmethylcolchicine in chronic granulocytic leukaemia (pp. 167, 179). While these drugs are effective in most cases, they do not appear to be superior to busulphan and are rather more difficult to manage satisfactorily. Their use in initial chemotherapy is not recommended, but either may reasonably be tried when a resistant state to busulphan develops.

Supportive treatment with blood transfusion to combat anaemia and antibiotics to deal with intercurrent infections, and the possible value of splenectomy in patients with refractory anaemia, a shortened red cell life-span and considerable splenomegaly, have also been discussed earlier (Chapter 11).

Examples of the practical management of therapy are given in the illustrative case reports at the end of this chapter.

Course and prognosis

The average survival from the first clear onset of symptoms is about 3 years, whether patients are treated or not. Survival data from many clinics are in general agreement, and from the reported case series it is clear that neither the introduction of X-rays nor changes in therapy over the past 30 years have led to any significant improvement in average survival. A selection of relevant figures, each derived from a substantial group of patients, with the average period of survival given in months, and the date of publication serving as a rough guide to the years in which each survey was made, illustrates this point. Minot, Buckman and Isaacs (1924), 41 months (untreated series); Hoffman and Craver (1931), 40 months; Leavell (1938), 38 months; Wintrobe and Hasenbush (1939), 40 months; Krebs and Bichel (1947), 42 months; Ledlie (1953), 36 months; Lawrence (1954), 39 months; Scott (1957), 32 months. It is too early to decide whether the widespread use of busulphan may alter the average survival, but present experience does not suggest that any very great change will be brought about.

Despite the constancy of average-survival data, very wide individual variations in survival occur. Tivey (1954), from a statistical analysis of longevity figures, calculated that 22 per cent of patients with chronic leukaemia, either granulocytic or lymphocytic, would survive more than 5 years from the time of onset, 6 per cent would live more than 10 years, and 0·8 per cent more than 20 years. Prognosis in the individual case is therefore most difficult, although young patients and those without gross physical signs at the time the diagnosis is made may be expected to live longer than the average, while elderly patients and those with marked splenomegaly, anaemia, and lymphadenopathy are likely to run a shorter course. Fever appears to be an unfavourable indication, especially if it persists during the early stages of treatment, and another ominous sign is the presence of a high proportion of myeloblasts and promyelocytes in the peripheral blood.

The commonest termination of chronic granulocytic leukaemia is by a myeloblastic transformation, the blood picture coming to resemble that of acute myeloblastic leukaemia with primitive cells predominating in the differential leucocyte count, and severe anaemia and thrombocytopenia rapidly developing. When this phase is reached, further remission can very rarely be achieved, although occasionally 6-mercaptopurine or demecolcine have been used with success. Even if remission can be induced, it is short-lived, and death may be expected to occur within a few weeks of the acute transformation.

reduced to below 10,000 cells per cu. mm. In practice, however, this ideal is difficult to achieve. Although the response to busulphan may remain uniform for long periods, a change in sensitivity to the drug may occur in either direction. If treatment is stopped, the white cell count may continue to fall to an unpredictable and varying extent.

Recommendations for routine use of busulphan (Hayhoe and Kok, 1957). The initial conservative dosage of 4 mgm. daily used by Galton and Till seems the most advisable regimen for the treatment of chronic myeloid leukaemia on an outpatient basis when blood counts and physical examination can be carried out at fortnightly to monthly intervals. Treatment should be stopped temporarily when the leucocyte count has fallen to between 10,000 and 20,000 per cu. mm., and recommenced at a lower level when the white cells have begun to increase. By this means a very gradual reduction to between 5,000 and 15,000 cells per cu. mm. may be effected with little risk of too severe a fall. Higher-dosage busulphan therapy in the occasional resistant patient is probably best carried out in hospital unless very frequent visits for blood counts can be made, since a sudden rapid fall may occur even in an initially resistant patient.

The average sensitive patient presenting a high peripheral leucocyte count may be given an initial dose of 10 mgm. a day when blood examinations at intervals of 2 to 3 days can be undertaken. This may be done, for example, in cases where treatment is begun in hospital. The busulphan should be stopped when the white count has fallen to about 25 per cent of the initial level, as recommended by Hyman and Gellhorn, unless the fall is very precipitous, in which case the drug should be stopped when the count has been roughly halved. When the leucocyte level has stopped falling, treatment may be started again with smaller doses, and an attempt made to reduce the level cautiously to about 10,000 cells per cu. mm. The rate of fall of the leucocytes during treatment is logarithmic, and if the total leucocyte count is plotted on a logarithmic scale a straight-line fall will usually be found on a constant dosage of busulphan. The probable result of continued treatment for a given period can therefore be roughly predicted by extrapolation on such a graph, and this information is most helpful in the control of dosage (see Case Reports and Figs. 43–46).

When the early stages of therapy have brought about a satisfactory fall in leucocyte count, improvement in haemoglobin level and in physical condition usually follows rapidly and a decision must then be made about maintenance treatment. It is unwise to continue giving busulphan, even in doses as low as 0·5 to 2 mgm. daily, to any patient whose leucocyte count is below 8,000 per cu. mm. When the white cell level has begun to rise, and is between 10,000 *and 20,000 per cu. mm., and even more so if it is higher, benefit is* likely to result from continued therapy. In general the aim should be to adjust dosage so that the count is kept between 10,000 and 20,000 cells per cu. mm. Because of the change in sensitivity which has already been mentioned, it is unsafe to leave a patient on treatment without blood examination for more than 4 weeks.

The onset of purpura or a sharp fall in platelet level at any stage of treatment should lead to prompt cessation of therapy, if it appears likely that the thrombocytopenia is drug-induced. If, however, haemorrhagic signs develop at a time when the leucocyte count is rising rapidly and the leukaemic process is escaping from control, increase in busulphan dosage may be successful in restoring both the leucocyte and platelet numbers towards normal. Concurrent administration of prednisone may help to reduce the bleeding tendency at these times.

prednisone was started in a dosage of 10 mgm. daily. An antiglobulin test was negative. Pyrexia and weight loss continued unchanged and busulphan was started once again when the leucocyte level had reached 80,000 per cu. mm. *A further period of good control* ensued, with disappearance of fever and hepatosplenomegaly, and increase in weight, on combined prednisone and busulphan, and 48 months after the first onset of symptoms (*42 months after treatment was started*) *the patient was still enjoying fair health.*

This case report illustrates a substantial period of control under busulphan therapy, and particularly the relation between busulphan dosage and the leucocyte level. On the logarithmic scale used in Fig. 43 it will be seen that continued even dosage of busulphan

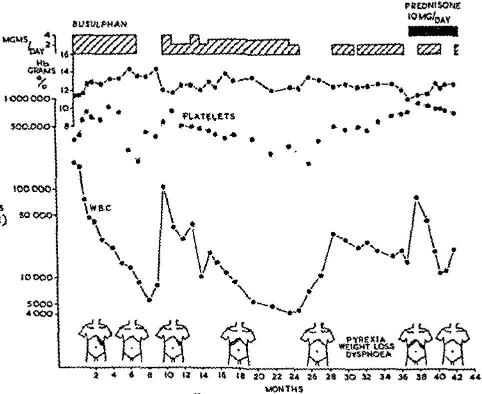


FIG. 43

was usually associated with an approximately straight-line fall in the leucocyte numbers, but it is noteworthy that alterations in sensitivity took place. Thus 2 mgm. daily of busulphan was insufficient to prevent a rise in white cell count during the 13th and 15th months of treatment, whereas the same dosage produced a steady shallow fall in the leucocyte level from the 28th to the 36th months and a steep fall from the 37th to the 40th months. The rate of fall during this last period on 2 mgm. of busulphan daily was in fact greater than that originally produced by twice the dosage.

Since the duration of life is not prolonged by either radiation or chemotherapy, the treatments available for chronic granulocytic leukaemia must still be regarded as highly unsatisfactory. Nevertheless, there is much to be gained from their use in terms of increased comfort and well-being during the remaining years of life. Untreated, the disease is nearly always inexorably progressive, with disabling anaemia and lassitude, steady weight loss and uncomfortable or even painful splenomegaly. Under treatment the patient commonly regains his normal sense of health and well-being and is able to continue his employment and enjoy his leisure for the greater part of his remaining life-span. When the final resistant state or myeloblastic transformation ensues, demise is not long delayed. The treated patient has therefore exchanged some years of increasing discomfort and illness for a period, almost equally long, of reasonably good health, followed by a short terminal illness. As in the case of acute leukaemia, there exists always the hope that continued active research may lead to the introduction of a new and more effective remedy during the years in which any patient is being kept under control, and the fact that the present methods of treatment are purely palliative and do not prolong the life-span lends a sharp spur to research efforts in this direction.

Illustrative Case Reports

CASE No. 7 (Fig. 43). Male, aged 67, was first diagnosed as having chronic granulocytic leukaemia in May 1955. He complained of steady loss of weight and increasing lassitude for the previous 6 months, the spleen tip was 10 cm. below the left costal margin, the haemoglobin level was 11·3 gm. per cent and the leucocyte count 200,000 per cu. mm. There was no enlargement of liver or lymph glands. Treatment with busulphan, 4 mgm. daily, produced a steady fall in the leucocyte count, which followed roughly a straight line when plotted logarithmically. The spleen became impalpable and the haemoglobin level gradually rose, reaching 14 gm. per cent after 6 months' treatment. The platelet count also rose initially from 350,000 to 700,000 per cu. mm., but fell to 200,000 per cu. mm. when the leucocyte count was reduced to 8,500 per cu. mm., 7 months after treatment was started. Busulphan was stopped at this stage, and during the next month the platelets rose to 450,000 per cu. mm. while the leucocyte count continued to fall, reaching 6,000 per cu. mm. Without maintenance treatment the leucocytes increased in number rapidly after this period of delayed busulphan effect, and 3 months after treatment had been stopped the leucocyte count was 100,000 per cu. mm., the haemoglobin had fallen to 12 gm. per cent and the spleen was again enlarged to 8 cm. below the costal margin. Treatment with busulphan was restarted and the subsequent fluctuations in haematological progress can be seen in Fig. 43. A substantial period of satisfactory control followed, but maintenance treatment was essential since the leucocyte count began to rise rapidly whenever treatment was stopped. During this time a haemorrhoidectomy was performed without untoward incident. Three years after the start of treatment there was an episode of pyrexia, dyspnoea, weight loss, and moderate enlargement of both liver and spleen without any obvious infection, and hospital investigations revealed no cause other than leukaemia. The possibility that busulphan might itself be responsible for the fever was considered and the drug was stopped for a month, and since the haemoglobin level was falling and there was a slight reticulocytosis suggesting a possible haemolytic element,

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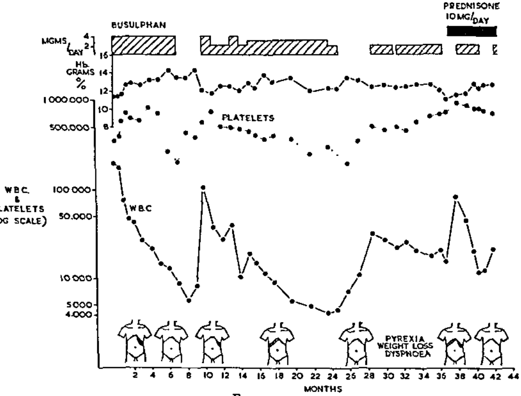

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admitted to hospital and treatment started with busulphan 4 mgm. daily, and his condition had improved enough within 2 weeks to allow him to be discharged for subsequent treatment as an outpatient. He has been satisfactorily controlled, remaining in good health and at work, for over 2 years, and no untoward incidents have occurred during this period. The haematological findings and the adjustments of busulphan dosage are shown in Fig. 44, from which the prediction value of a graph having a logarithmic scale for the leucocyte count can again be seen.

This case report illustrates a typical response to busulphan, with gradual reduction in leucocyte count to the required level, and later attempts to establish a satisfactory maintenance dose. The majority of patients treated with busulphan respond in a similar way, control being achieved without difficulty and maintained for a substantial period without serious problems.

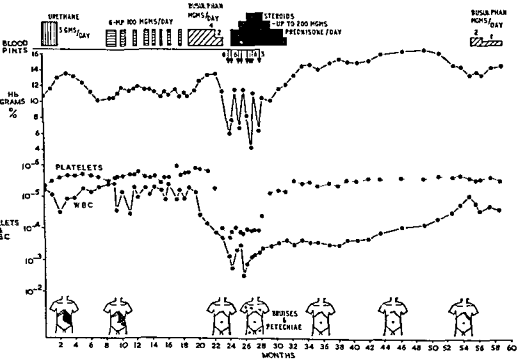

FIG. 45.

CASE NO. 9 (Fig. 45). A woman aged 67 was admitted to hospital in January 1954 with typical chronic granulocytic leukaemia. She had been increasingly unwell since April 1953, with muscular pains in the legs, backache, lassitude, constipation, and abdominal swelling. Her spleen extended across the midline and almost to the pelvic brim, and the liver edge was palpable 5 cm. below the right costal margin. Examination of the peripheral blood showed haemoglobin 10·8 gm. per 100 ml. and leucocytes 230,000 per cu. mm., with 46 per cent granulocyte precursors. Platelets numbered 300,000 per cu. mm. Treatment

The continuation of maintenance treatment with 3 mgm. and later 2 mgm. daily between the 18th and 24th months, when the leucocyte count was below 10,000 per cu. mm., had no ill effects on this patient, but experience with other patients suggests that such continuation was in fact unwise, since there is a definite risk of marrow aplasia being produced (see Case No. 9).

CASE No. 8 (Fig. 44). Male, aged 50 years, was first diagnosed as having chronic granulocytic leukaemia in December 1956. His presenting symptoms were lancinating pains in

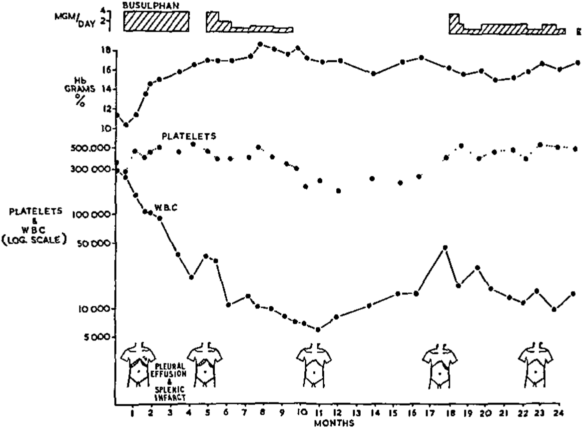

FIG. 44.

the left shoulder, followed by pain in the left subcostal region during respiration, and he was found to have signs of splenic infarction and a small associated left basal effusion. At the same time venous thrombosis led to sudden and painful testicular swelling. There was moderate enlargement of both liver and spleen, but no palpable lymphadenopathy. A blood count revealed the haemoglobin level to be 11·4 gm. per cent, the platelets 350,000 per cu. mm. and the leucocyte count 290,000 per cu. mm. with a typical differential pattern, namely, 33 per cent segmented neutrophils, 18 per cent stab cells, 20 per cent metamyelocytes, 13 per cent myelocytes, 9 per cent promyelocytes, 2·5 per cent myeloblasts, 1 per cent eosinophils, 2·5 per cent basophils, 1 per cent lymphocytes. He was

admitted to hospital and treatment started with busulphan 4 mgm. daily, and his condition had improved enough within 2 weeks to allow him to be discharged for subsequent treatment as an outpatient. He has been satisfactorily controlled, remaining in good health and at work, for over 2 years, and no untoward incidents have occurred during this period. The haematological findings and the adjustments of busulphan dosage are shown in Fig. 44, from which the prediction value of a graph having a logarithmic scale for the leucocyte count can again be seen.

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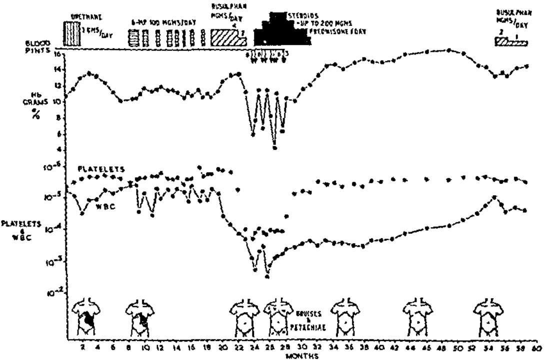

FIG. 45.

CASE No. 9 (Fig. 45). A woman aged 67 was admitted to hospital in January 1954 with typical chronic granulocytic leukaemia. She had been increasingly unwell since April 1953, with muscular pains in the legs, backache, lassitude, constipation, and abdominal swelling. Her spleen extended across the midline and almost to the pelvic brim, and the liver edge was palpable 5 cm. below the right costal margin. Examination of the peripheral blood showed haemoglobin 10·8 gm. per 100 ml. and leucocytes 230,000 per cu. mm., with 46 per cent granulocyte precursors. Platelets numbered 300,000 per cu. mm. Treatment

was started with 1 gm. of urethane three times a day, and was continued for 2 months before it had to be stopped because of increasingly severe nausea. During this time the leucocyte count fell to 40,000 per cu. mm. and the haemoglobin level rose to 13 gm. per 100 ml. The spleen had become smaller and the general clinical state was much improved. During the next 6 months a gradual deterioration in the blood count took place, but the patient felt well and was symptom-free. Bone and muscle pains then began to recur, and both the splenic size and the peripheral-blood picture had reverted to the pretreatment position. Therapy was started again, this time with mercaptopurine (100 mgm. daily). Response to mercaptopurine was rapid, but lasted only a few weeks after the drug had been stopped, and several short courses of treatment were therefore given during the succeeding 10 months.

Because of the fluctuating haematological findings, and the unstable clinical course on mercaptopurine therapy, treatment with busulphan was started on July 14th, 1955, in a dosage of 4 mgm. daily. The response was very good, with a steady rise in haemoglobin and fall in leucocyte count, and a reduction in spleen and liver size, so that after 104 days the haemoglobin had reached 13·4 gm. per 100 ml., the leucocytes numbered 8,200 per cu. mm., with no granulocyte precursors present, platelets were 200,000 per cu. mm., and neither liver nor spleen could be felt. At this stage the dosage of busulphan was reduced to 2 mgm. daily.

One month later the leucocyte count had fallen to 3,750 per cu. mm., while the haemoglobin had dropped to 11·2 gm. per 100 ml. and platelets had decreased sharply to about 10,000 per cu. mm. There were no specific complaints or fresh physical signs, and, in particular, no sore throat or purpura. Busulphan was stopped but pancytopenia became increasingly severe, and 4 weeks later, on December 23rd, there were scattered petechiae and well-marked bruises on the limbs, and the blood count showed haemoglobin 6 gm. per 100 ml., leucocytes 1,600 per cu. mm., and platelets fewer than 10,000 per cu. mm. The patient was admitted to hospital and treated with transfusions of fresh blood and courses of cortisone, corticotrophin, and prednisone. For a period of 4 months there was no sign of returning haemopoietic activity, the haemoglobin level fluctuating in accordance with transfusions, the white cells varying between 350 and 3,000 per cu. mm., with relatively few polymorphs, and the platelets remaining fewer than 10,000 per cu. mm.

The first clear evidence of commencing recovery appeared on May 3rd, 1956, when the expected fall in haemoglobin level during the week after a transfusion did not take place and reticulocytes rose to 2·9 per cent. The reticulocytosis persisted between 3 and 7 per cent for the next 2 months, and during this time the blood findings all showed clear improvement. Steroid therapy was stopped on June 5th and subsequent progress was uninterrupted. A very full clinical and haematological remission ensued, with high haemoglobin and platelet levels and a leucocyte count that remained stable in the region of 4,000 per cu. mm. for some 8 months and then rose very slowly, reaching 10,000 after 16 months and 100,000 after 24 months. By this time the haemoglobin level had begun to fall once more and busulphan was again administered. The patient still responded to the drug and the condition was again controlled.

This case illustrates successively the problem of nausea complicating urethane treatment, and preventing its continuance, the difficulty of maintaining stable control by intermittent courses of 6-mercaptopurine, the risk of medullary aplasia if treatment with

busulphan, even in low dosage, is continued after the leucocyte count has fallen below 10,000 per cu. mm., the use of high-dosage steroid therapy during an aplastic phase, and the very prolonged remission following recovery from a drug-induced aplastic state. The patient was still surviving nearly 6 years after the first onset of symptoms.

CASE No. 10 (Fig. 46). Female, aged 23 years, was first seen in August 1955, with a complaint of easy bruising, noticeable during the previous 6 weeks. Examination revealed

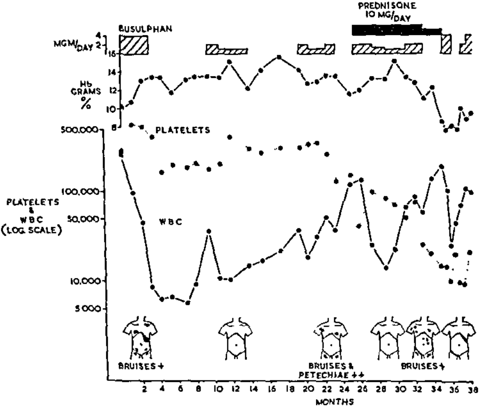

FIG. 46.

scattered ecchymoses but no petechiae, the spleen was palpable, extending 10 cm. below the left costal margin, but neither the liver nor the superficial lymph glands were enlarged. A blood count showed the characteristic picture of chronic granulocytic leukaemia, with haemoglobin 10·2 gm. per cent, platelets 260,000 per cu. mm. (despite the haemorrhagic tendency), and leucocytes 250,000 per cu. mm. with 60 per cent granulocyte precursors. Treatment was started with busulphan 4 mgm. daily, and the subsequent progress can be seen from Fig. 46. After nearly 2 years of satisfactory control, a recurrence of bruising with multiple skin petechiae, a subconjunctival haemorrhage, and bleeding from the oral mucosa, took place, this time associated with a sharp fall in the platelet count. The

thrombocytopenia might have been due to busulphan therapy, but it developed at a stage when the leucocyte count was rising between 20,000 and 100,000 per cu. mm., and might therefore more probably be a result of leukaemic cell proliferation. The second possible cause appeared still more likely when 2 months without busulphan did not lead to any improvement, but rather deterioration in the platelet level and haemorrhagic state. Busulphan was therefore restarted, in combination with prednisone to minimize capillary leakage, and a further satisfactory clinical remission took place, accompanied by a rise in haemoglobin, a fall in leucocyte count and some improvement in platelet level. After 10 months, bruising again appeared, and busulphan was once again stopped, treatment being continued with prednisone alone for 6 weeks. The haemorrhagic state deteriorated during this period, but control was once more achieved with busulphan alone.

This case illustrates the occasional haemorrhagic onset of chronic granulocytic leukaemia, the achievement of control under busulphan treatment, and the difficult problem of thrombocytopenic bleeding developing during the course of treatment. Whereas in Case No. 9 such bleeding was undoubtedly part of a generalized bone-marrow aplasia, in the present case it was associated with a rising leucocyte count, and must therefore have been due either to a selective thrombocytopenogenic effect of busulphan, or to leukaemic proliferation with secondary thrombocytopenia, unrelated to therapy except in so far as therapy was inadequate to control the proliferation. Responses to further courses of busulphan, given despite the thrombocytopenia, suggest that the second explanation holds in this case.

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thrombocytopenia might have been due to busulphan therapy, but it developed at a stage when the leucocyte count was rising between 20,000 and 100,000 per cu. mm., and might therefore more probably be a result of leukaemic cell proliferation. The second possible cause appeared still more likely when 2 months without busulphan did not lead to any improvement, but rather deterioration in the platelet level and haemorrhagic state. Busulphan was therefore restarted, in combination with prednisone to minimize capillary leakage, and a further satisfactory clinical remission took place, accompanied by a rise in haemoglobin, a fall in leucocyte count and some improvement in platelet level. After 10 months, bruising again appeared, and busulphan was once again stopped, treatment being continued with prednisone alone for 6 weeks. The haemorrhagic state deteriorated during this period, but control was once more achieved with busulphan alone.

This case illustrates the occasional haemorrhagic onset of chronic granulocytic leukaemia, the achievement of control under busulphan treatment, and the difficult problem of thrombocytopenic bleeding developing during the course of treatment. Whereas in Case No. 9 such bleeding was undoubtedly part of a generalized bone-marrow aplasia, in the present case it was associated with a rising leucocyte count, and must therefore have been due either to a selective thrombocytopenogenic effect of busulphan, or to leukaemic proliferation with secondary thrombocytopenia, unrelated to therapy except in so far as therapy was inadequate to control the proliferation. Responses to further courses of busulphan, given despite the thrombocytopenia, suggest that the second explanation holds in this case.

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CHAPTER 14

CHRONIC LYMPHOCYTIC LEUKAEMIA: CLINICAL ASPECTS

CHRONIC lymphocytic leukaemia is more varied than the chronic granulocytic form both in its modes of presentation and in its clinical course. The findings in the peripheral blood and bone marrow are readily diagnostic in most cases, however, since they reveal a great increase in the numbers of mature lymphocytes. Just as, in a small proportion of cases with chronic granulocytic leukaemia, confusion may exist with non-leukaemic myeloproliferative states, so, in the chronic lymphocytic disease, atypical or borderline forms may be difficult to differentiate from non-leukaemic proliferative disorders of lymphocytic tissues, especially lymphosarcoma and to a lesser extent Hodgkin's disease, lymphoid follicular reticulosis and other lymphomatous states. Indeed, there is undoubtedly an ill-defined borderland zone between typical chronic lymphocytic leukaemia and the more common forms of malignant lymph-gland tumours, in which neither the haematological and pathological findings nor the clinical features enable a certain allocation to be made to one group or the other. The relation of lymphocytic leukaemia to these "lymphoproliferative" states is discussed in Chapter 16, and we shall be concerned for the present with the clinical aspects of unequivocal chronic lymphocytic leukaemia.

The rising incidence of this variety of luekaemia with increasing age and the preponderance of males among those affected have already been described in Chapter 3. The disease is rare before middle age but becomes the commonest form of leukaemia in the elderly.

Modes of onset

The onset is even more insidious than that of chronic granulocytic leukaemia, and chance discovery of the disease as a result of routine blood examination during the investigations of some apparently unrelated disorder is quite common (Hougie, 1956). Scott (1957) reported that 13·2 per cent of 212 patients with chronic lymphocytic leukaemia had been first diagnosed in this way, although other signs of the disease were often to be found at this stage when carefully sought. Of the patients whose symptoms first draw attention to the possibility that they may be suffering from lymphocytic leukaemia, most complain of enlargement of the lymph glands, especially those in the neck. In Scott's series this was the presenting symptom in 42·5 per cent; other presenting symptoms in order of frequency were lassitude (in 24·5 per cent), loss of weight (in 9 per cent), symptoms of anaemia (in 9 per cent), skin lesions (in 7 per cent), abdominal pain (in 6·6 per cent), angina (in 5·7 per cent), spontaneous haemorrhages (in 5·2 per cent), consciousness of an abdominal tumour (in 4·7 per cent) and ankle oedema (in 3·8 per cent).

Repeated minor infections are a common feature of the disease, both in the early stages, when they may lead to the discovery of the leukaemic blood picture, and throughout the whole course. Reference has been made earlier (p. 201) to the defective capacity for antibody formation and the decrease in gamma-globulins sometimes shown by patients

with chronic lymphocytic leukaemia, and the recurrent infections are presumably attributable to this defect in resistance. Septic lesions of the skin, upper respiratory tract infections, and urinary infections are especially common.

The physical signs found at the first examination are less uniform than in granulocytic leukaemia, but over 70 per cent of patients have generalized moderate enlargement of the lymph nodes, usually with palpable but not gross splenomegaly and hepatomegaly. Pallor is not often conspicuous. A considerable minority of patients, between 10 and 20 per cent, show a close resemblance to the typical clinical picture of granulocytic leukaemia, having massive enlargement of the spleen, a moderate degree of hepatomegaly, and slight or absent superficial lymphadenopathy (Plate XXVII, Fig. 4). Occasionally the enlargement of lymph glands may be localized, affecting a single node or group of nodes and clinically resembling Hodgkin's disease or lymphosarcoma. Rarely purpura and haemorrhagic signs or leukaemic skin infiltrations may be present without other physical signs of the disease. When the leukaemic blood picture is discovered by chance during the investigation of infection or other disease states, there may be no abnormal physical signs directly attributable to leukaemia.

System involvement and correlated pathology

Lymph glands. Generalized enlargement of lymph glands is a prominent and early feature of chronic lymphocytic leukaemia (Plate XXVII, Figs. 1 and 2). Huge gland masses are uncommon, and the enlarged nodes tend to remain discrete, so that the usual finding is of lymph nodes from 1 to 5 cm. in diameter, palpable in considerable numbers in all accessible sites. The glands are firm and painless. Those in the cervical chains are most often noticed by the patient, but axillary and inguinal lymphadenopathy is usually found on examination and mesenteric nodes may be felt. Enlargement of mediastinal glands can often be detected by radiography and is sometimes the cause of respiratory or venous obstruction, or other pressure effects. Comparable hypertrophy of lymphocytic tissues elsewhere in the body occurs, and enlargement of the tonsils and adenoids may be conspicuous. The intestinal Peyer's patches are commonly found at autopsy to be much swollen, and rarely polypoid masses of lymphocytic tissue may project into the lumen of the gut.

When sectioned, the lymph glands present a homogeneous greyish surface with little sign of haemorrhage or necrosis. Histologically, the normal architecture of the gland cannot be discerned, the follicular pattern being replaced by a diffuse even sheet of leukaemic lymphocytes. The capsule remains intact, without malignant invasion, and the predominating cells are uniform mature lymphocytes which do not show the variability in cell size and the frequent mitotic figures seen in the more anaplastic forms of lymphosarcoma. Microscopic areas of necrosis or haemorrhage are uncommon. While the typical appearances are clearly distinguishable from those of a highly malignant lymphosarcoma, in a considerable proportion of cases there exists sufficient lack of cellular uniformity and sufficient tendency to capsular invasion to make the differential diagnosis difficult or impossible on the glandular histology alone (Gall and Mallory, 1942).

The Mikulicz syndrome of bilaterally symmetrical painless swelling of the salivary and lachrymal glands may occur as a result of lymphocytic infiltration, and although uncommon, this complication has long been recognized (Rowe, 1930; Hird, 1949). The lachrymal

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The Mikulicz syndrome of bilaterally symmetrical painless swelling of the salivary and lachrymal glands may occur as a result of lymphocytic infiltration, and although uncommon, this complication has long been recognized (Rowe, 1930; Hird, 1949). The lachrymal

gland enlargement tends to produce raised intra-orbital pressure and proptosis, while involvement of the salivary glands may lead to dryness of the mouth and sometimes to auditory defects.

Spleen. Splenomegaly is slight or moderate in most cases of chronic lymphocytic leukaemia, but, as we have already seen, gross enlargement comparable to that usually found in granulocytic leukaemia is present in rather less than a fifth of all patients. Infarction, subcapsular haemorrhage and perisplenitis are frequent complications when the splenic enlargement extends to the umbilicus or below, but seldom occur with minor degrees of splenomegaly. The appearance of the spleen at autopsy has no definitive characteristics. The general shape is preserved and the consistency unremarkable. Microscopy reveals replacement of the normal splenic pattern of Malpighian bodies, pulp and sinuses by a homogeneous lymphocytic infiltrate, made up of mature cells, with little capsular invasion. So uniform is the cellular infiltration that material aspirated by splenic puncture during life is composed almost entirely of lymphocytes (Moeschlin, 1951), and if the percentage of lymphocytes in a splenic smear from an undiagnosed patient is less than 90, chronic lymphocytic leukaemia is unlikely to be present. The majority of the cells are small mature lymphocytes, but varying numbers of prolymphocytic forms, having the cytological characteristics of similar cells in the blood and bone marrow, are also to be seen.

The liver. Hepatic enlargement is moderate or occasionally gross, and at post-mortem examination the organ appears pale with often a macroscopically visible infiltrate of greyish-white tissue. Histological sections reveal dense accumulations of lymphocytes in the portal tract regions, with a less conspicuous infiltrate along the hepatic sinusoids (Plate XXVII, Figs. 5, 6 and 7). Larger nodules of leukaemic cells are sometimes found, and these, or glandular masses in the porta hepatis, may obstruct the free flow of bile and lead to jaundice. Ascites may develop in the terminal stages, probably as a result of several factors, including decreased serum proteins and raised portal venous pressure secondary to extensive hepatic involvement.

The skin. Cutaneous manifestations are common in chronic lymphocytic leukaemia. Epstein and MacEachern (1937) found skin lesions of one variety or another in 46·6 per cent of patients with the disease, some having more than one type of lesion at the same time. They regarded 8·3 per cent as having specific skin infiltration with leukaemic cells, the remainder having non-specific associated phenomena such as purpura and ecchymoses (25 per cent), pruritis (3·3 per cent) and, less commonly, macular, maculo-papular, pustular or vesicular rashes, or herpes zoster. The specific skin lesions do not conform to a regular pattern, but may be localized, chiefly to the face, or generalized over most of the body surface (Plate XXVII, Fig. 3). They vary from a barely discernable localized reddish thickening of the skin to gross generalized exfoliative erythrodermia, and an enormous range of papular, nodular, macular and haemorrhagic leukaemic infiltrates has been reported.

The majority of specific skin lesions in chronic lymphocytic leukaemia probably arise as "cutaneous metastases", which proliferate independently once formed (Gates, 1938). Certainly, the skin infiltrations usually develop after the generalized changes of leukaemia have become well established. In a small proportion of cases, however, the skin rash appears before the leukaemia has become manifest elsewhere, and it is possible that in

some of these cases the disease may originate from the lymphocytic and reticulo-endothelial elements of the skin and subsequently spread to involve secondarily the lymph glands, spleen, marrow and blood.

A rare but supposedly characteristic thickening and distortion of the nose, eyebrows and ears, with the production of gross facial disfigurement, has been observed several times to precede the development of leukaemic changes in the blood (Sweitzer, 1916; Butler, 1920). Another variety of skin rash sometimes preceding the onset of frank lymphocytic leukaemia and usually pursuing a mild and chronic course for several years is generalized leukaemic erythrodermia or "leukaemia cutis universalis". In this condition the entire skin is reddened and thickened ("homme rouge") with accentuation of the skin folds, and usually little desquamation. Occasionally atrophic changes may develop and marked desquamation may accompany loss of body hair. During the course of the disease the lymph glands and spleen become enlarged and specifically leukaemic changes are found in the peripheral blood and bone marrow. Cases were described by Sequeira and Panton (1921, 1925) and subsequently by various authors (MacCormac and Whitby, 1937; Holten, 1952). The rash may begin anywhere on the body but spreads gradually until it has become universal. There is commonly severe pruritis. The peripheral leucocyte count has not usually been greatly raised even in fully developed cases, ranging generally between 8,000 and 30,000 per cu. mm , but some 60 to 90 per cent of the cells are mature lymphocytes. Skin sections show extensive infiltration of the dermis with small lymphocytes and occasional eosinophils and macrophages. At post-mortem examination the internal organs show changes characteristic of lymphocytic leukaemia.

Herpes zoster occurs much more commonly in chronic lymphocytic leukaemia than can be explained by chance association. Scott (1957) found herpes zoster present at the beginning of the illness in 7 of 212 patients, and during the course of their disease probably at least 5 per cent of patients develop herpes. In many of these cases the skin lesions start in the form of a localized vesicular rash in the cutaneous area of distribution of a cranial or spinal nerve, but later become generalized, with a disseminated varicelliform eruption. Wile and Holman (1940) collected 34 examples of such generalized dissemination, and further reports have confirmed the existence of a tendency for herpes zoster to spread in leukaemic patients (Barton and O'Leary, 1945; Rodnan and Rake, 1956). The generalized rash usually appears within a week of the initial herpetic lesions, rapidly reaches maximum intensity and fades before the primary rash. Neither the high incidence of the primary herpes zoster lesions, nor the tendency to dissemination observed in patients with chronic lymphocytic leukaemia has been adequately explained. Craver and Haagensen (1932) suggested that leukaemic infiltration of afferent portions of the reflex arc might predispose to infection, but this possibility remains unproven. As to the generalized spread of the vesicular rash, this occurs so rapidly as to suggest a haematogenous rather than a neurotrophic method of dissemination (Feyrter, 1954; Rodnan and Rake, 1956), and spread may be facilitated by the immunological impairment existing in lymphocytic leukaemia. When the skin lesions have eventually healed, there may be residual loss of sensation in the area, or, more commonly, severe and intractable post-herpetic neuralgia, which may persist throughout the whole remaining course of the leukaemia.

Bones and joints. Skeletal lesions gross enough to give rise to clear localizing symptoms or to radiological changes are far less common in the chronic than in the acute

leukaemias. Nevertheless, Craver and Copeland (1935) found radiological bone changes in 7 per cent of 86 patients with chronic lymphocytic leukaemia, and some, though not all, of the affected patients had local pain, tenderness or swelling. As Scott (1957) has pointed out, the most characteristic skeletal lesion in chronic lymphocytic leukaemia is diffuse decalcification with progressive osteoporosis, affecting most conspicuously the lower dorsal and lumbar regions of the vertebral column. Vertebral collapse may develop and compression of the spinal cord with paraplegic symptoms and signs sometimes occurs. Osteoporotic changes may be seen also in radiographs of the pelvis, long bones and skull, but pathological fractures involving these bones are rare. Localized areas of bone destruction due to circumscribed nodules of leukaemic tissue may rarely be seen, and subperiosteal new bone formation is sometimes observed. The generally high age incidence of the lymphocytic form of leukaemia is such that reactive bone responses to infiltration are slight and degenerative changes predominate.

Pathologically, the active proliferation and infiltration of leukaemic lymphocytes within the bone marrow of all parts of the skeleton leads to erosions and breakdown of the bony trabeculae in the spongy zones, from which marrow can be scooped out at autopsy with no difficulty. At the same time expansion of the marrow cavity occurs, as in other types of leukaemia, at the expense of the cortical layer, which appears obviously thinned and porotic.

Specific lesions of the joints with involvement of synovial membranes are uncommon, but juxta-articular bony lesions with extensive destruction and deformity and secondary arthritic changes have, rarely, been observed.

It remains uncertain whether the widespread use of corticosteroids in chronic lymphocytic leukaemia may lead to an increase in skeletal osteoporosis and pathological fractures as a result of steroid demineralizing activity. The lymphocytolytic actions of the corticosteroids may, however, compensate for any demineralizing effect by reducing lymphocytic infiltration, and the nett result of their use may be a reduction in osteoporosis.

Central nervous system. Histological examination of material obtained *post mortem* shows the presence of haemorrhagic or infiltrative lesions in the nervous system in most cases of chronic lymphocytic leukaemia. Reference has already been made to paraplegic or other spinal cord syndromes resulting from vertebral collapse. Serious neurological disorders may arise from haemorrhages into the cerebrum, brain-stem or elsewhere, consequent upon thrombocytopenia, especially in the terminal stages of the illness. Apart from these somewhat indirect neurological effects of leukaemia, involvement of the nervous system is rarely important clinically. Occasionally paralysis of cranial nerves may be seen, the 7th and 6th nerves being most often affected, while a wide range of neurological phenomena, from meningism and coma to regional paraesthesiae or paralyses, has been recorded. In this respect there appears little difference between the varieties of leukaemia, neurological disorders due to direct infiltration of the central nervous system being relatively infrequent in all of them (Schwab and Weiss, 1935).

Eyes and ears. Retinal changes, with venous engorgement, haemorrhages and exudates, occur much less commonly and strikingly in lymphocytic than in chronic granulocytic leukaemia or the acute leukaemias, but such changes may occasionally be seen in terminal thrombocytopenic phases of the disease. Nodular collections of leukaemic cells in the cornea, iris or sclera are more common than in other forms of leukaemia, although

still quite rare. They may lead to pain in the eye and disturbance of vision and have sometimes been the first indication of disease (Wintrobe and Mitchell, 1940). Infiltration of the middle or inner ear, or of the 8th nerve, causing Ménière's syndrome or deafness, has been reported infrequently. Attention has earlier been drawn to possible ocular and aural symptoms associated with infiltration of the lachrymal and parotid glands, when Mikulicz's syndrome develops. All these infiltrative lesions affecting the eyes and ears are uncommon. Haemorrhagic lesions in these sites, apart from occasional retinal haemorrhages, are equally rare except in the terminal stages when bleeding into the vitreous, conjunctiva, or auditory apparatus may occur as part of a generalized secondary thrombocytopenic purpura.

Gastro-intestinal system. Symptoms attributable to infiltration or leukaemic nodule formation in the alimentary tract are seldom encountered, although minor disturbances of appetite and bowel habits are common enough as in most serious systemic diseases. In the later stages of chronic lymphocytic leukaemia haemorrhage may occur from the gastro-intestinal tract, and gastric or duodenal ulceration is sometimes found at autopsy (Case No. 14). Terminal thrombocytopenia probably initiates bleeding from mucosal erosions or ulcers. Very rarely massive infiltration of parts of the alimentary canal has been observed, with widespread thickening of the mucosa and submucosa from extensive infiltration with lymphocytes. The hypertrophied inner layers of the gut are then thrown into exaggerated folds and convolutions, and pedunculated lymphomatous polyps may be found (Rigler, 1936; Pearson, Stasney and Pizzolato, 1943). Gross invasion of this sort is quite uncommon in patients with the usual blood picture and clinical findings of lymphocytic leukaemia, but a number of examples of "pseudoleukaemia gastro-intestinalis", in which extensive lymphocytic infiltration and hypertrophy of the gut has been found in the absence of typically leukaemic changes elsewhere, have been reported (Boikan, 1931; Mead, 1933).

Although macroscopic evidence of infiltration is not often conspicuous at post-mortem examination, an occasional polypoid nodule is to be seen not uncommonly in the stomach, large intestine, rectum, or less frequently in the small intestine. Histologically, infiltrating leukaemic lymphocytes are almost invariably present in the mucosal and submucosal layers throughout the gut.

Genito-urinary system. The chief genito-urinary complications of chronic lymphocytic leukaemia are inflammatory or haemorrhagic ones. The pronounced susceptibility to infections shown by patients with this form of leukaemia leads to the frequent occurrence of cystitis or pyelitis, often due to *Escherichia coli*, and generally responding satisfactorily to sulphonamides or appropriate antibiotics. When thrombocytopenia develops, bleeding from either the renal tract or the female genital tract commonly occurs. Haematuria may occur in the absence of infection or thrombocytopenia as a result of severe infiltration and congestion of the kidneys, or as a consequence of tubular obstruction and damage from uric acid deposits derived from excessively rapid breakdown of leucocytes under aggressive radiotherapy or chemotherapy. Infiltration of the kidneys, prostate, ovaries and uterus tends to be more marked in lymphocytic than in the other varieties of leukaemia, and these organs are often macroscopically pale and swollen at autopsy. The age incidence of chronic lymphocytic leukaemia is such that a proportion of male patients might be expected to suffer from benign hypertrophy of the prostate, and

leukaemic infiltration probably exacerbates any existing tendency to urinary obstruction. For this reason a uraemic termination of the disease may take place. Attempts to relieve urinary obstruction by prostatectomy at a late stage are rarely successful, the combined risks of post-operative infection and haemorrhage being very great, but the operation may be safely performed in the early stages of the illness when the platelet count is still high and immunological resistance to infections has not yet been severely depressed.

Uraemia may also develop in either sex as a result of gross infiltration of the kidneys with leukaemic tissue or with amyloid deposits. Scott (1957) reported the occurrence of a nephrotic syndrome in two patients with lymphocytic leukaemia. Both showed high levels of gamma-globulins in the serum and had heavy albuminuria, and developed progressive oedema and renal failure. In one case autopsy revealed amyloid deposits in the kidneys, liver and myocardium, and in the other marked renal infiltration with lymphocytes. Examination of the peripheral blood and bone marrow showed the changes of chronic lymphocytic leukaemia, with small mature cells predominating, and in neither case was there any cytological evidence of myelomatosis or plasma-cell leukaemia.

Respiratory system. Falconer and Leonard (1938) found significant pulmonary or mediastinal involvement in about 30 per cent of patients with chronic lymphocytic leukaemia, and certainly at least a third of all patients develop symptoms attributable to enlarged mediastinal lymph nodes or to pulmonary or pleural infiltration with consolidation or pleural effusion. Huge mediastinal gland masses leading to acute respiratory obstruction are seldom encountered, but the bronchial obstruction often resulting from smaller masses is frequently complicated by added infection with broncho-pneumonic changes, and while these may respond to antibiotics they sometimes prove terminal. Patterns of miliary infiltration of the lung parenchyma or of multiple leukaemic nodules have occasionally been reported, but such widespread pulmonary involvement is very unusual (Herold and Michel, 1940; Scott, 1957). Equally rare is leukaemic infiltration of the larynx.

Apart from enlargement of lymph nodes and infective or haemorrhagic changes in the respiratory tract, post-mortem examination usually reveals signs of pleural and pulmonary infiltration, if not macroscopically, at least when histological sections are studied. The frequent finding of serous pleural effusions is evidence of the tendency for leukaemic cells to invade the pleura, especially in the later stages of the disease.

Cardiovascular system. Anaemia developing during the course of chronic lymphocytic leukaemia in elderly patients with some degree of pre-existing coronary atheroma readily precipitates attacks of angina pectoris, and this exacerbation of myocardial ischemia is sometimes the presenting symptom. When anaemia and anoxemia are severe, cardiac failure, with dyspnoea, venous engorgement and peripheral oedema, may develop.

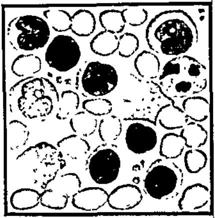
Specifically leukaemic involvement of the cardiovascular system is rarely so prominent as to cause symptoms or signs during life, although some areas of infiltration in the myocardium can be found almost invariably when histological examination is carried out *post mortem*. Pericardial infiltration is not common, but an increase in pericardial fluid may be present.

Findings in the blood and bone marrow (Plate XXVI)

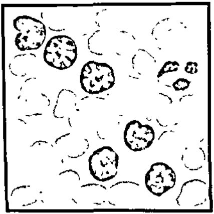
The blood. The appearance of the peripheral-blood smear in chronic lymphocytic leukaemia is usually quite diagnostic. The leucocytes are increased in numbers, often

PLATE XXVI

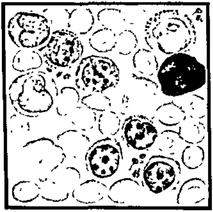
CYTOLOGY AND CYTOCHEMISTRY OF CHRONIC LYMPHOCYTIC LEUKAEMIA



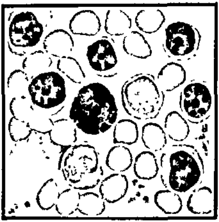
1. May-Grunwald-Giemsa stain



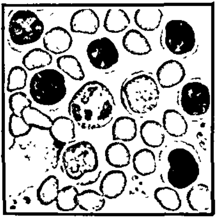
2. Feulgen reaction



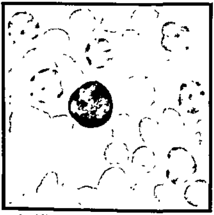
3. Periodic acid-Schiff reaction



4. Sudan black B stain



5. Peroxidase reaction



6. Alkaline phosphatase reaction

PLATE XXVII

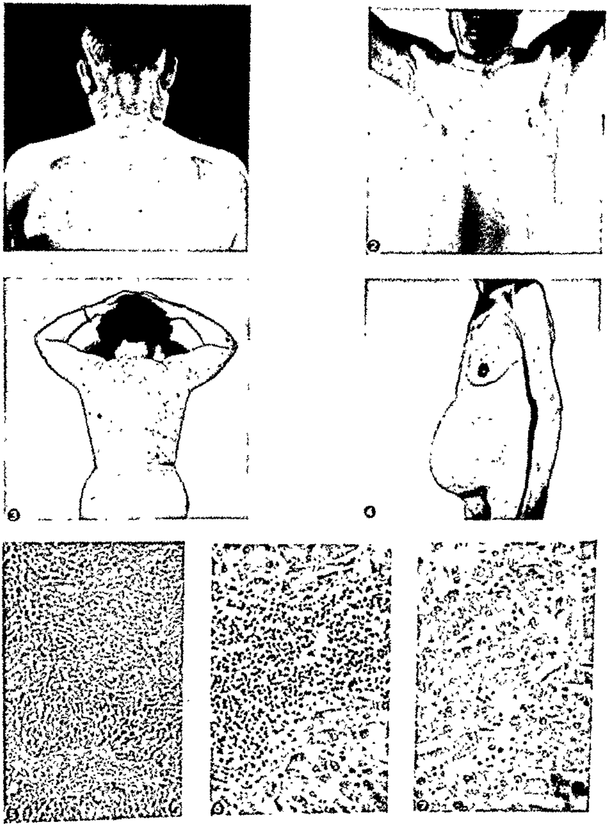
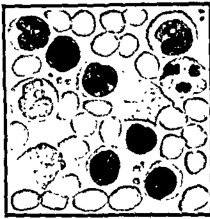
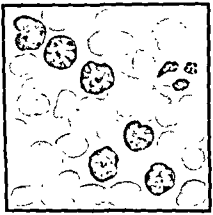


PLATE XXVI

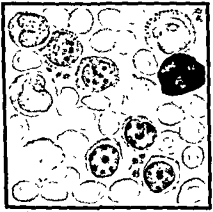
CYTOLOGY AND CYTOCHEMISTRY OF CHRONIC LYMPHOCYTIC LEUKAEMIA



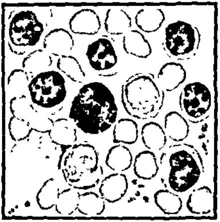
1. May-Grunwald-Giemsa stain



2. Feulgen reaction

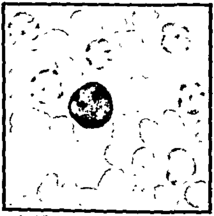


3. Periodic acid-Schiff reaction



4. Sudan black B stain

5. Peroxidase reaction



6. Alkaline phosphatase reaction

To face p 368

diagnosed. At this stage the red cells appear normochromic and normocytic, but as the leukaemia progresses, hypochromia and anisocytosis develop and the haemoglobin falls. The nature of the anaemia has been discussed in Chapter 11, where it has been made clear that either latent or overt haemolysis, often with auto-immune sensitization of the red cells and a positive anti-globulin test, is frequently present in chronic lymphocytic leukaemia. When this is the case, macrocytosis, anisocytosis and sometimes spherocytosis may be seen, the serum bilirubin is slightly raised, and reticulocytosis develops, with polychromasia in Romanowsky-stained blood films. The haemolytic syndrome usually develops slowly and the marrow retains sufficient regenerative capacity to hold the haemoglobin level between 8 and 12 gm. per cent, but much more active haemolysis, quite beyond the compensatory reserve of the marrow, sometimes occurs, leading to rapid and drastic reduction of the red cells and haemoglobin to dangerous levels.

Thrombocytopenia is not prominent throughout the greater part of the course in most cases of chronic lymphocytic leukaemia, although it commonly develops terminally, usually as part of an aplastic syndrome of marrow failure, with accompanying failure of red cell production. In a small proportion of cases, however, thrombocytopenia may be present during the earlier stages of the disease, sometimes in association with a frankly haemolytic anaemia, and it is tempting to speculate that the reduction in platelet numbers in the circumstances may be due to the action of platelet auto-antibodies comparable to the red cell auto-antibodies responsible for haemolysis. The recognition of possible immune mechanisms in the genesis of anaemia and thrombocytopenia in chronic lymphocytic leukaemia is especially important since effective treatment for these complications is available in the corticosteroid hormones.

The bone marrow. Smears of aspirated bone marrow frequently show a marked increase in the relative proportion of lymphocytes, which make up from 30 to 80 per cent of all the nucleated marrow cells. The cytological characters of the infiltrating lymphocytes are similar to those seen in the peripheral blood, and features of immaturity are not more conspicuous in the marrow. Occasionally there may be only slight increase, or none at all, in the proportion of marrow lymphocytes in an aspirated sample, even when the clinical findings and those in the peripheral blood are diagnostic. It seems probable that, in many cases, lymphocytic proliferation may not extend to involve the marrow generally or uniformly for some time after the disease has developed in primarily lymphocytic sites. Very much less commonly extensive lymphocytic infiltration of the bone marrow may be found when the lymph glands and spleen are not enlarged and the peripheral-blood changes not clearly diagnostic (Storti, 1937). While in such exceptional cases sternal puncture is a valuable diagnostic procedure, it is unnecessary in the vast majority of cases, where the physical signs and peripheral-blood findings leave the diagnosis in no doubt.

A fairly common variant of the usual predominantly lymphocytic pattern of marrow cytology is that seen in cases having a strong element of haemolysis due to auto-antibody activity. Here, erythroblastic proliferation, with macronormoblastic cytology, develops alongside the leukaemic lymphocytopoiesis, producing a mixed pattern of lymphocytic and erythroblastic increase. When haemolysis is controlled by corticosteroid therapy, erythropoietic activity recedes and lymphocytes predominate alone once more.

Megakaryocytes are usually to be found in normal numbers and with unimpaired thrombocytopoietic activity throughout most of the course of the disease. When thrombo-

greatly, and the vast majority of them are clearly members of the lymphocyte series. Total leucocyte counts at the time of initial examination, before treatment has been started, generally range between 20,000 and 200,000 per cu. mm., but higher or lower counts occur in a substantial minority of patients. Very high counts, between 500,000 and a million per cu. mm., are quite exceptional, but figures below 10,000 per cu. mm. are met with in about one patient in ten. Whatever the total figure, the differential count reveals an overwhelming preponderance of lymphocytes, which constitute from 80 to 99 per cent of all nucleated cells present. The cytological characters of the lymphocytes vary considerably from case to case and it is very unusual to find an entirely uniform pattern even in a single blood-smear. Most often small lymphocytes, with a densely staining pachychromatic nucleus and a narrow rim of pale-blue cytoplasm, not differing greatly from normal small lymphocytes in reaction to panoptic stains, are dominant, but the mechanical fragility of many of these cells is increased and "smear cells" are usually present, often in large numbers, especially in the tail and at the edges of the film. A variable percentage of the lymphocytes show "prolymphocytic" features, with a larger, more leptochromatic, nucleus and a greater amount of cytoplasm, which may contain vacuoles or azurophil granules and has sometimes an increased basophilia. Lymphoblasts, with a large primitive, nucleolated nucleus and a small amount of basophilic cytoplasm are rarely seen at any stage of the disease, except in very small numbers, and a terminal lymphoblastic phase, parallel to the myeloblastic reversion of chronic granulocytic leukaemia, is quite uncommon. Close cytological study of the lymphocytes in chronic lymphocytic leukaemia reveals, as we have seen earlier (Chapter 5), no distinctively leukaemic features in individual cells. While small lymphocytes, intermingled with occasional cells having prolymphocytic features, and accompanied by a considerable proportion of disrupted smear cells, provide the common peripheral-blood leucocyte picture, occasionally large lymphocytes, not unlike those occurring in normal blood, though often with indented irregular nuclei, and rather filmy and indefinite cytoplasmic outlines, may predominate. There appears to be little relationship between the clinical course to be expected in any case of the disease and the cytological pattern of the peripheral blood, whether predominantly large or small cell, mature or less mature.

Cytochemically, notable features are the increased content of glycogen in leukaemic lymphocytes and the normal or high level of alkaline phosphatase activity in the polymorphonuclear cells (see Chapter 5).

Anaemia tends to occur later in the disease than in granulocytic leukaemia and the majority of patients have haemoglobin levels above 10 gm. per cent when they are first

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PLATE XXVII

CHRONIC LYMPHOCYTIC LEUKAEMIA

Clinical and Histological Features

1 and 2. Cervical and axillary lymphadenopathy and enlargement of the spleen at the time of first clinical examination in a patient with chronic lymphocytic leukaemia.
3. Extensive lymphocytic skin infiltrates in a patient without blood or marrow lymphocytosis.
4. Gross splenomegaly in chronic lymphocytic leukaemia.
5, 6 and 7. Pattern of hepatic infiltration, predominantly portal, in chronic lymphocytic leukaemia. (× 25, × 100, × 100)

biopsy reveals lymphocytic hyperplasia without the malignant features of lymphosarcoma or the histological pattern of Hodgkin's disease or other non-leukaemic reticulosis. These uncommon cases are sometimes referred to as "lymphocytic lymphoma without leukaemia", but they usually develop characteristically leukaemic blood changes in the course of time and the diagnosis becomes clear retrospectively. When glandular enlargement precedes the development of leukaemic changes in the peripheral blood, and especially when a single group of glands is primarily affected, the differentiation between lymphosarcoma and chronic lymphocytic leukaemia may be impossible. There is, indeed, no sharp distinction between these two diseases, which, though different enough in their typical manifestations, share a common and ill-defined border zone clinically and pathologically. Discussion of the interrelationships of these and other members of the lymphoproliferative group of diseases will be found in Chapter 16.

Treatment

Many cases of chronic lymphocytic leukaemia, particularly in elderly patients but also in some younger ones, pursue a remarkably benign and protracted course. These are often patients in whom the blood abnormality is discovered incidentally during the course of some minor illness or infection, and as far as the leukaemia is concerned they may remain free from symptoms for many years. In these circumstances no treatment is required until progress of the disease produces definite disability. Whereas, in chronic granulocytic leukaemia, symptoms are severe enough from the time of first diagnosis to demand treatment in nearly every case, and active therapy should always be instituted at once, there is need for a careful decision, whether to start treatment or not, when chronic lymphocytic leukaemia is diagnosed. The best general guide is in the patient's symptomatic complaints. If he feels well, has not been losing weight, can work normally and eats and sleeps well, there is nothing to be gained from early treatment even if the blood picture shows a high lymphocytosis, and minor degrees of lymphadenopathy and hepatosplenomegaly can be detected. Without treatment, such patients often lead normal lives for periods of 2 to 10 years before increasing enlargement of lymph glands, liver and spleen, accompanied often by progressive anaemia, lassitude, weight loss and heightened susceptibility to infection, makes active therapy advisable. When symptoms attributable to leukaemia are present from the time of first medical examination, treatment should not be delayed.

Radiotherapy for long remained the chief form of treatment in chronic lymphocytic leukaemia after its introduction half a century ago. It is certainly effective in reducing the size of lymph-gland masses, and radiation is commonly directed against the groups of glands showing most prominent enlargement or giving rise to pressure symptoms. The radiosensitivity of gland masses varies rather widely, and successive doses of 50 to 100 r may be given at intervals of 2 to 3 days until a satisfactory effect is achieved. A total dose of 1,000 r usually proves enough to give resolution of any single mass of glands, but the general clinical state, the haemoglobin level, and the lymphocyte count in the peripheral blood are rarely much affected. Irradiation of other localized lesions, such as areas of bone erosion with vertebral collapse, provides an excellent local response in many cases, again without altering the general features of the disease. The peripheral leucocyte count may be reduced by splenic irradiation, and therapy is commonly directed against both the spleen and the enlarged glands, but the g[illegible]ect is sometimes frustrating, since the

cytopenia develops in the peripheral blood, one of two opposite pictures is to be found in marrow aspirates. In the first and more common, occurring chiefly in the later stages of the disease, very few megakaryocytes can be seen. The thrombocytopenia is an "amegakaryocytic" one, usually part of a more general medullary hypoplasia with accompanying failure of erythropoiesis and granulopoiesis. In the second marrow pattern, there are normal numbers of megakaryocytes, but defective platelet formation, as in idiopathic thrombocytopenic purpura. Such a "megakaryocytic" thrombocytopenia is perhaps due to auto-immune mechanisms and may co-exist with haemolytic anaemia.

When examined *post mortem*, the marrow throughout the skeleton generally resembles that in chronic granulocytic leukaemia as far as macroscopic appearances are concerned, with replacement of both normal red marrow and inactive fatty marrow by greyish-red hyperplastic tissue. Thinning of the cortical bone and irregular destruction of trabeculae are also seen. Sections reveal a comparable picture to that seen in aspirates, with a great preponderance of densely packed mature lymphocytes, smaller numbers of prolymphocytic cells, and separated clumps of normal haemopoietic tissue. Rarely, when death has occurred from intercurrent infection or some other cause in the relatively early stages of disease development, bone-marrow involvement may be slight, the patterns of both macroscopic marrow appearances and microscopic differential cytology being comparatively normal.

Differential diagnosis from non-leukaemic states

The clinical and haematological picture of chronic lymphocytic leukaemia is sufficiently typical in most cases for problems of differential diagnosis rarely to arise. The discovery of lymphadenopathy, perhaps associated with hepatosplenomegaly, at preliminary physical examination, raises many possibilities, including tuberculosis, Hodgkin's disease, follicular lymphoma, lymphosarcoma and reticulosarcoma as well as leukaemias of various kinds. The tendency for the glands in tuberculosis to be soft or fluctuant and to become attached to the skin, for those of Hodgkin's disease and follicular lymphoma to belong initially to one or two groups only and to remain firm and discrete, and for those of the sarcomas to invade locally, forming large masses, helps the initial clinical differentiation, but, if leukaemia is present, doubts are usually set rapidly at rest by examination of the peripheral blood, since substantial lymphocytosis is to be found in few non-leukaemic states. Other disorders in which peripheral lymphocytosis may reach levels over 20,000 per cu. mm. include glandular fever, pertussis and infectious lymphocytosis (Smith, 1941). All these conditions occur chiefly in children or young adults and are rare among the older age-groups where chronic lymphocytic leukaemia has its greatest incidence. Glandular fever may be further differentiated by the cytology of its characteristic lymphomononuclear cells and by the Paul-Bunnell sheep's cell agglutination test; pertussis by its clinical picture, since the only resemblance between whooping-cough and lymphocytic leukaemia lies in the lymphocytic blood changes; and infectious lymphocytosis by its feverish onset, association with gastro-intestinal infection, and benign course with early recovery. These disorders enter more into the differential diagnosis of blood films than of the complete disease picture.

More difficult problems arise when there is enlargement of lymph glands, spleen and liver, but no convincing evidence of leukaemia in the blood or bone marrow, and gland

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lymph glands and spleen decrease in size and the lymphocyte count falls but the haemoglobin fails to rise and the patient's general state of health is not greatly improved (Leavell, 1938; Scott, 1949). A more satisfactory result, with improvement in anaemia and clinical condition as well as reduction in lymphadenopathy, occurs in about 50 per cent of patients after their first course of deep X-ray. Responses last variable periods, but further treatment is usually necessary within a year, and second and subsequent courses become decreasingly effective. Radioactive phosphorus may be used in treatment, with the advantages and disadvantages compared with X-rays that have previously been mentioned (Chapter 9), but, as in the case of chronic granulocytic leukaemia, chemotherapy offers a more acceptable alternative to X-rays and radioactive phosphorus is now very seldom used for the routine management of patients with chronic lymphocytic leukaemia.

Among the chemotherapeutic substances useful for the control of lymphocytic leukaemia the alkylating agents are by far the most effective, while corticotrophin or corticosteroids have a special contribution to make in certain cases with haemolytic and thrombocytopenic complications. Reference has been made in Chapter 10 to the range of available alkylating agents, and to earlier drugs such as arsenic and urethane, which were formerly used in the treatment of chronic leukaemias. Their modes of action, dosages and toxic effects were discussed. Both arsenic and urethane were shown to be unreliable and attended by seriously disadvantageous side-actions. Of the more effective alkylating agents, those which have found a place in the routine chemotherapy of chronic lymphocytic leukaemia during the past 10 years are the nitrogen mustards, triethylene melamine, and chlorambucil, and since the last of these was introduced, its comparative ease of handling and freedom from side-effects have made it unquestionably the chemotherapeutic agent of choice. Very satisfactory results have been claimed from the use of either nitrogen mustards or triethylene melamine by those thoroughly familiar with their potentialities and toxic effects, but the variability in individual sensitivity always makes dose calculation difficult. Moreover, the nitrogen mustards frequently cause distressing nausea and vomiting and have the further disadvantage of requiring careful intravenous injection, while triethylene melamine is rather irregular and unpredictable in its action and likely to produce serious thrombocytopenia and perhaps marrow aplasia in certain very sensitive patients even when used in orthodox dosage.

Chlorambucil has little tendency to induce nausea or other unpleasant side-effects, and its action can be brought to bear in a more gradual and effectively controlled manner than is possible with the previous agents. The risks of aplasia are therefore greatly reduced.

In the practical management of chronic lymphocytic leukaemia, when symptoms requiring treatment develop, the most appropriate method is chemotherapy with chlorambucil, the dosage being adjusted to reduce the leucocyte count gradually to between 10,000 and 20,000 per cu. mm. and to maintain it in this region by intermittent courses or low-dosage maintenance therapy. When this is done, most patients show a parallel improvement in clinical condition, with decrease in size of lymph glands, spleen and liver, and sometimes, though rather less often, a rise in haemoglobin level. Radiotherapy has no advantage over chlorambucil for general treatment, but may conveniently be applied to achieve rapid resolution of localized glandular masses causing pressure symptoms, or

to arrest focal bone erosion. It is wise to avoid the combined use of chlorambucil and radiotherapy unless the latter is being given in very small doses, since there is an increased risk of medullary aplasia when the two cytotoxic effects summate.

When anaemia persists after control of the leucocyte count and reduction in lymphadenopathy and hepatosplenomegaly have been brought about by chlorambucil or radiation, a trial of prednisone in low dosage (5 to 20 mgm. daily) is always worth while, even if there is no clear evidence of overt haemolysis and the antiglobulin test is negative. In the majority of patients some improvement in haemoglobin level ensues, and in many, especially those with frank auto-immune haemolytic anaemia, the improvement is remarkable. The continued use of corticosteroids in patients with chronic lymphocytic leukaemia has disadvantages in view of the great susceptibility to infections shown by many of them, and it is therefore wise to reduce the prednisone dosage to a minimum compatible with satisfactory maintenance of haemoglobin level. Indeed, in some cases treatment can be stopped entirely for long periods without recurrence of anaemia (Case No.12). A further indication for trial of prednisone is the development of thrombocytopenia, whether alone or as part of an aplastic phase. In the former case there is more likelihood of satisfactory response, but even in marrow aplasia corticosteroids may help to stimulate renewed haemopoiesis and assist recovery.

As in all other forms of leukaemia, striking and prolonged remission sometimes follows chemotherapy so aggressive as to induce partial marrow failure (Case No. 13). The deliberate use of doses large enough to cause aplasia is not, of course, justifiable, since one cannot guarantee to induce a reversible as distinct from an irreversible aplasia, but the experience of occasional accidental overdosage in unusually sensitive patients emphasizes the potential value of aggressive therapy if it could be pushed to the margin of aplasia and no further, or if transplantation techniques could ensure recovery.

Illustrations of the use of different therapeutic agents in the control of various phases of chronic lymphocytic leukaemia are given in the case-histories at the end of this chapter. It is not possible to make any simple recommendation for the routine dosage adjustments when chlorambucil is used, since there is so much variation in sensitivity of different patients and circumstances so often call for added corticosteroid therapy or deep X-ray treatment to local lesions. As suggested in Chapter 10, an initial dosage of about 0·1 mgm. per kg. body weight is customarily used, and clinical and haematological examinations carried out at intervals of 1 to 2 weeks, the dosage being varied to achieve a progressive improvement in leucocyte count and physical state. Too rapid a fall in lymphocytes is to be avoided, since urinary obstruction may occur with very rapid breakdown of cells and excessive excretion of uric acid, as has been described earlier when the treatment of chronic granulocytic leukaemia with busulphan was discussed. Chlorambucil, again like busulphan and other cytotoxic agents, continues to reduce haemopoietic activity for a week or two after oral administration has been stopped, and allowance for some continuation of the fall in leucocyte level must be made when deciding that the time for discontinuance of the drug has come.

The frequency of repeated courses or the need for maintenance therapy must be assessed in each individual case according to the rate at which relapse occurs. Wide variability occurs in this regard, much more so than in chronic granulocytic leukaemia. Some patients show signs of clinical relapse and a rising lymphocyte count in the peri-

pheral blood within 2 or 3 weeks of finishing an apparently effective course of treatment, whereas others remain in substantial remission for months or even years.

In view of their tendency to develop infections, patients with chronic lymphocytic leukaemia must be warned to seek medical advice early if symptoms develop, especially in the respiratory or urinary tracts. Appropriate treatment with antibiotics or other means can then be instituted as soon as possible and protracted infection avoided.

Course and prognosis. The variability in individual longevity after diagnosis in chronic lymphocytic leukaemia is even greater than that in chronic granulocytic leukaemia. Many patients, in whom the disease has been discovered incidentally before onset of leukaemic symptoms, continue for several years before symptoms appear, whereas others, whose disease is widespread and fulminating when they are first examined, may die within weeks or months. There is less unanimity, too, about average survival data, partly because of discrepancies in different case series over the inclusion of patients with lymphosarcoma and "leukaemic" blood changes. Minot and Isaacs (1924) reported mean survival from onset to be 40 months in 30 untreated patients and 42 months in 50 treated by X-rays. Over 30 years later, despite all advances in treatment, Scott (1957) found the average survival of 118 patients to be 36·7 months. It is clear, therefore, that treatment has not increased the duration of life in this disease. Rather greater average survival figures, from 4 to 5 years or more, have been reported from some centres where cases of possible "lymphosarcoma cell leukaemia" have been carefully excluded (Lawrence, 1954; Sturgis, 1955).

Apart from difficulties of classification, the value of average survival data is rendered still smaller by the impossibility of determining, even within a wide span of months, the date of onset of the disease. Symptoms arise so insidiously that patients can often not recall when they first began to feel unwell, although it is common for symptoms to have been present certainly for more than a year before diagnosis is made (Wintrobe and Hasenbush, 1939). Very probably, blood examination would reveal changes in the average patient at least 2 or 3 years before the onset of symptoms, and possibly much longer, to judge from experience with cases discovered by chance.

While the mean survival from the time of first clear onset of symptoms is between 3 and 5 years, the prognosis in an individual patient may diverge very widely from the mean. Survival periods of 8 years or more after diagnosis are quite common, and occasional examples of survival over 20 years have been reported, the longest being one of 29 years (Marlow and Bartlett, 1953). Since chronic lymphocytic leukaemia affects principally elderly patients, a proportion of them may be expected to die from causes other than leukaemia, such as cardiovascular disease or carcinoma, and a further proportion succumb to infections, such as broncho-pneumonia, to which there may exist a predisposition arising from the defective immune response in this form of leukaemia. Longer than average survivals are to be expected in patients with minimal physical signs and only moderately elevated leucocyte counts at the time of diagnosis, whereas those with florid clinical pictures and high leucocyte counts are likely to deteriorate more rapidly than the average.

The course of chronic lymphocytic leukaemia, once clear-cut symptoms have emerged, is inexorably downhill. In the early stages deterioration tends to be slow and treatment is often effective in controlling and perhaps temporarily reversing it, but after a variable

period progressive and intractable anaemia, not responding to treatment with either anti-leukaemic drugs or corticosteroids, develops, usually accompanied by a haemorrhagic state and often by severe infection of the respiratory tract or elsewhere, and death soon follows. An increase in cells with lymphoblastic features in the peripheral blood may sometimes occur in the late stages of the disease, but this is not a common or characteristic finding and a frankly lymphoblastic termination comparable to the myeloblastic termination often seen in chronic granulocytic leukaemia is extremely rare.

What has been said earlier about the value of treatment in chronic granulocytic leukaemia applies largely to the treatment of chronic lymphocytic leukaemia as well. Here again, no definite prolongation of life can be offered, but the comfort, well-being and useful activity of the patient during the remaining years of his life after disabling symptoms first appear are very much improved by appropriate therapy. Moreover, here too there is always hope that a more effective remedy may be discovered during the period of temporary control afforded by currently available agents.

Illustrative Case Reports

CASE No. 11 (Fig. 47). Female, aged 48 years, was admitted to hospital in July 1949 for investigation and treatment of menorrhagia and prolapse. Blood counts revealed the expected iron-deficiency anaemia, with haemoglobin level 7 gm. per cent and mean corpuscular haemoglobin concentration 26 per cent, but, unexpectedly, the peripheral-blood leucocyte pattern of chronic lymphocytic leukaemia was also found to be present, with 40,000 leucocytes per cu. mm., predominantly small lymphocytes but with occasional prolymphocytic features. There were no physical signs suggestive of leukaemia, and in particular the lymph glands, liver and spleen were not palpably enlarged, but a sternal marrow examination showed 36 per cent of the nucleated cells to be lymphocytes. Treatment with oral iron was started when the gynaecological disorder had been corrected surgically and an excellent response was achieved. Since the leukaemic state was causing no symptoms, treatment directed against the leukaemia was not given. Blood counts and routine physical examinations were performed at intervals over the next 8 years without disclosing any evidence of deterioration, although the leucocyte level in the peripheral blood remained between 20,000 and 40,000 per cu. mm. The haemoglobin level fell gradually during the second and third years to 11 gm. per cent, but rose again slowly to 14 gm. per cent after a further prolonged course of oral iron. Eight years after the diagnosis was first made, there was an onset of pain and weakness in the leg muscles, with marked paraesthesiae in the feet and legs. There were signs of peripheral neuritis, with loss of ankle jerks and defects of sensation, and bilateral papilloedema was also found. Lumbar puncture revealed an excess of lymphocytes in the cerebro-spinal fluid and an increase of protein, while the Queckenstedt test showed a slow rise and fall of pressure. The Wassermann reaction was negative and the Lange colloidal gold curve normal. It was thought possible that the disseminated neurological signs might be due to scattered infiltration of the meninges by leukaemic cells, and a course of chlorambucil, in a dosage of 6 mgm. daily, was given for a period of 2 months. The leucocyte count fell during this time from 60,000 to 14,000 per cu. mm., the papilloedema slowly cleared, and power and sensation returned in the legs. Vitamin B_1 supplements were then also given, and the

leucocyte count was maintained between 10,000 and 25,000 per cu. mm. by intermittent courses of chlorambucil, 2 mgm. daily, during the next 18 months. The clinical condition remains satisfactory, with only slight residual symptoms in the legs. At no stage throughout the 10 years of clinical observation has this patient shown palpable enlargement of lymph glands, liver or spleen.

This case report illustrates the unexpected discovery of chronic lymphocytic leukaemia in a patient investigated because of an apparently unrelated condition and having no physical signs attributable to leukaemia. The case also typifies the prolonged benign course, without need for treatment, followed for several years by many patients with this disease. A very unusual neurological complication, with papilloedema and peripheral

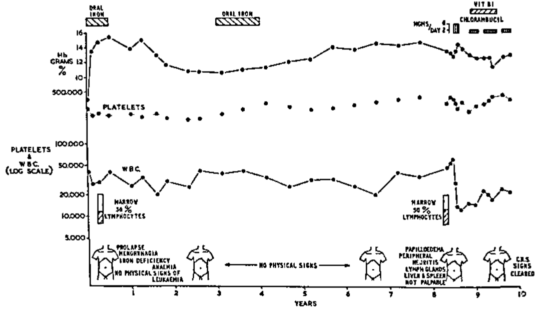

FIG. 47.

neuritis, presumably due to disseminated meningeal infiltrates, responded satisfactorily to chemotherapy with chlorambucil.

CASE NO. 12 (Fig. 48). Male, aged 45 years, first sought medical advice in February 1956 because of enlargement of lymph glands in the neck. Examination revealed gross lymphadenopathy at all palpable sites, the gland masses measuring up to 10 cm. in diameter, while the spleen extended 10 cm. below the umbilicus and the liver edge was felt 8 cm. below the right costal margin. Peripheral blood counts showed the leucocytes to number between 70,000 and 80,000 per cu. mm., most of them being lymphocytes, and a sternal marrow aspirate and lymph-gland biopsy confirmed the diagnosis of chronic lymphocytic leukaemia. A course of radiotherapy to the cervical and axillary glands reduced their size and brought the leucocyte count down rather rapidly to 9,400 per cu. mm., and there was

a short clinical remission during which the haemoglobin level, which had been falling steadily before and during treatment, rose a little, and the hepato-splenomegaly also diminished. In less than 3 months, however, relapse took place and treatment with chlorambucil was therefore started, initially in a dosage of 8 mgm. daily, reduced after a week to 6 mgm. daily. The leucocyte count gradually fell, reaching 9,000 per cu. mm. after 5 weeks, and treatment was then stopped. Unfortunately the haemoglobin fell during this time to 6 gm. per cent, the platelet count also fell, and the physical signs showed only moderate improvement. A transfusion of 4 pints of blood was given and

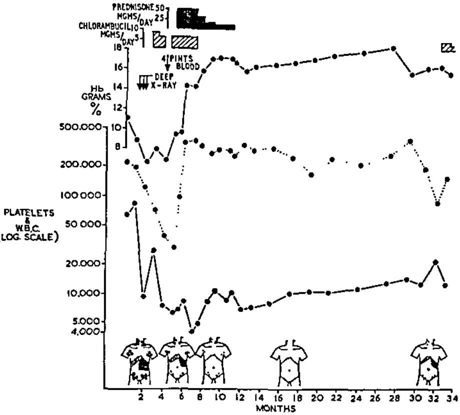

Fig. 48.

chlorambucil, 6 mgm. daily, was restarted 3 weeks later when the leucocyte count again began to rise. Although the anaemia was not clearly haemolytic, there being no icterus and the reticulocytes numbering less than 2 per cent, a trial of prednisone was thought worthwhile to combat anaemia and thrombocytopenia. This drug was therefore given in combination with chlorambucil, the dosage initially being 50 mgm. daily. A spectacular improvement followed, with the development of a remarkably complete clinical and haematological remission, and after 10 weeks' combined therapy the haemoglobin had risen to 14·2 gm. per cent, and the platelets to 350,000 per cu. mm., the leucocyte count had fallen to 4,000 per cu. mm. and the only abnormal physical findings were a few small palpable glands. Chlorambucil was stopped at this stage and the prednisone gradually

tailed off over the next 3 months. Full remission was maintained for nearly 2 years before recurrent lymphadenopathy and enlargement of liver and spleen, with falling haemoglobin, necessitated fresh treatment.

This case-history illustrates a florid disease picture at the time of first examination, a temporary and partial period of control brought about by radiotherapy to the enlarged gland masses, a reduction in leucocyte count unaccompanied by general remission achieved by chlorambucil, and a remarkable improvement induced by a combination of prednisone and chlorambucil. The severe anaemia and thrombocytopenia did not recur *for 2 years after all treatment had been stopped.*

CASE No. 13 (Fig. 49). Male, aged 61 years, was admitted to hospital in May 1951 with a complaint of increasingly severe cough with profuse sputum and breathlessness in the

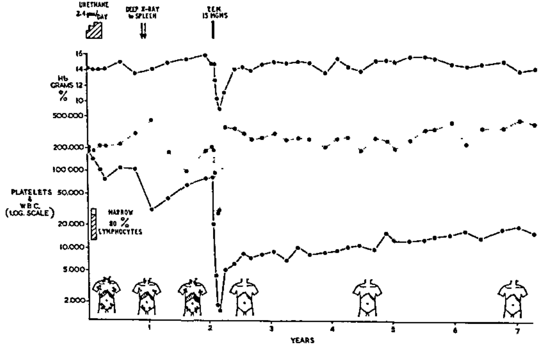

FIG. 49.

previous 3 months. He had lost 20 lb. in weight in the past year and had recently developed angina of effort. Examination revealed moderate enlargement of the spleen (4 cm. below the left costal margin) and of the liver (6 cm. below the right costal margin), and large soft mobile glands were felt in the neck, axillae and groins. The peripheral-blood count showed a picture diagnostic of chronic lymphocytic leukaemia, with haemoglobin 14·2 gm. per cent, and leucocytes 190,000 per cu. mm., the majority being small lymphocytes. There were many smear cells present. The platelets numbered 200,000 per cu. mm. A sternal marrow aspirate revealed a very marked lymphocytic infiltration, with 80 per cent

lymphocytes and 3·5 per cent lymphoblasts. In addition to the leukaemia, he was found to have right basal bronchiectasis and coronary atheroma, and treatment with antibiotics and coronary artery dilator drugs was prescribed when necessary during his subsequent course.

The leukaemia was treated initially with urethane, 2 to 4 gm. daily, and the leucocyte count fell and the clinical condition improved, but nausea became intolerable and the treatment was stopped after 3 months. He remained well, with the glands, liver and spleen somewhat smaller, for the next 6 months, but then began to suffer from pain in the splenic region, and a short course of radiotherapy to the spleen was given. The leucocyte count again fell and there was temporary relief of pain, but symptoms recurred and the physical signs returned to the original state after a year. At this stage treatment with triethylene melamine (TEM) was given, 5 mgm. daily for 3 days, and within a week the leucocyte count had fallen from 80,000 to 4,000 per cu. mm., the platelets had dropped to 30,000 per cu. mm. and blotchy purpura appeared on both legs. During the following month there was persistent pancytopenia, with fall in haemoglobin to 9 gm. per cent, leucocyte levels between 1,000 and 2,000 per cu. mm. and severe thrombocytopenia, but at the same time the lymphadenopathy and hepta-splenomegaly regressed very markedly. A very full remission then ensued, the blood picture returning almost to normal, though still with some preponderance of lymphocytes, and the only physical sign attributable to leukaemia was just palpable enlargement of the spleen. This state of affairs has persisted for the last 5 years, during which no further anti-leukaemic therapy has been required, although there have been recurrent episodes of bronchitis and treatment has been needed for angina of effort.

This case report illustrates again the diagnosis of leukaemia in a patient admitted for the investigation of non-leukaemic complaints, but here there were gross physical signs of the disease. The difficulty of maintaining adequate urethane dosage in the face of severe nausea and the temporary effect of a short course of radiotherapy are described, and the remarkable response to a rather high dose of TEM, with a phase of pancytopenia followed by prolonged clinical and haematological remission, is shown. This patient remains well, at age 68, more than 7 years after the discovery of a florid clinical and haematological chronic lymphocytic leukaemia.

CASE No. 14 (Fig. 50). Male, aged 51 years, was first seen in November 1952 complaining of sore throat and swelling of the neck glands for the previous 3 weeks. He had lost a few pounds in weight in the past month. His previous health had been good apart from a peptic ulcer which had perforated 12 years before but which had given rise to no more than occasional slight indigestion since then. The only abnormal physical findings were slight enlargement of the cervical, axillary and inguinal glands and considerable swelling and inflammation of the tonsils. Neither liver nor spleen could be felt. A blood count showed the picture of chronic lymphocytic leukaemia, with haemoglobin 13·2 gm. per cent, platelets 320,000 per cu. mm., and leucocytes 59,000 per cu. mm., 80 per cent of them being small lymphocytes. A sternal marrow examination revealed extensive infiltration with lymphocytes which made up 78 per cent of the nucleated cells. Chest X-ray showed prominence of both hilar regions and signs of partial consolidation in the right mid-zone, thought to be due to lymphatic obstruction from enlarged glands in the right hilum. The

tailed off over the next 3 months. Full remission was maintained for nearly 2 years before recurrent lymphadenopathy and enlargement of liver and spleen, with falling haemoglobin, necessitated fresh treatment.

This case-history illustrates a florid disease picture at the time of first examination, a temporary and partial period of control brought about by radiotherapy to the enlarged gland masses, a reduction in leucocyte count unaccompanied by general remission achieved by chlorambucil, and a remarkable improvement induced by a combination of prednisone and chlorambucil. The severe anaemia and thrombocytopenia did not recur for 2 years after all treatment had been stopped.

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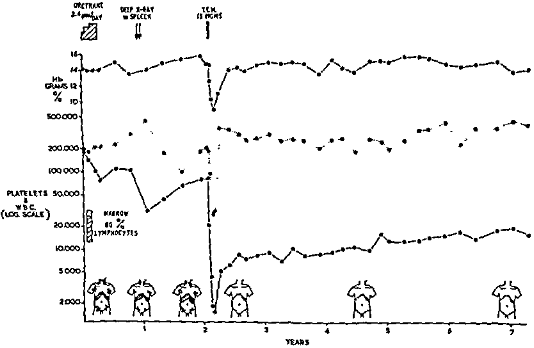

FIG. 49.

previous 3 months. He had lost 20 lb. in weight in the past year and had recently developed angina of effort. Examination revealed moderate enlargement of the spleen (4 cm. below the left costal margin) and of the liver (6 cm. below the right costal margin), and large soft mobile glands were felt in the neck, axillae and groins. The peripheral-blood count showed a picture diagnostic of chronic lymphocytic leukaemia, with haemoglobin 14·2 gm. per cent, and leucocytes 190,000 per cu. mm., the majority being small lymphocytes. There were many smear cells present. The platelets numbered 200,000 per cu. mm. A *sternal marrow aspirate revealed a very marked lymphocytic infiltration*, with 80 per cent

with successful short-term reductions in gland size, but the leucocyte count showed only transitory decreases and control of the general disease process was not satisfactory, since further glandular enlargement developed elsewhere as soon as one group of glands had been reduced by X-rays. Radiotherapy was therefore discontinued and no treatment given during the fourth year of the illness since at this time the patient felt well enough to continue working normally despite considerable lymphadenopathy. The haemoglobin level fell gradually over this period to 11 gm. per cent and the leucocyte count climbed to 650,000 per cu. mm., and at the end of the year numerous glands, 2 to 4 cm. in diameter, were palpable at all superficial sites and the spleen had enlarged to 4 cm. below the left costal margin. Platelets had decreased to 100,000 per cu. mm. but there were no bleeding signs.

Treatment with chlorambucil was now started and continued, with a single rest period of 2 months, for the final 2 years of the disease. The dosage given initially was 8 mgm. daily, and subsequent variations in dosage in relation to the leucocyte count can be seen in Fig. 50. The haematological response was excellent for over 18 months, with rise in haemoglobin and platelets and fall in leucocytes to less than 20,000 per cu. mm. Lymphadenopathy and splenomegaly were also reduced although numerous small glands could still be felt. After 16 months' treatment with chlorambucil a rest period was given to allow the normal haemopoietic activity of the marrow to recover from prolonged cytotoxic effects and to see whether remission would continue without maintenance therapy. The leucocyte count began to rise rapidly, however, and although it was again brought under control by chlorambucil, a steadily progressive anaemia developed and finally thrombocytopenia with bleeding from the gastro-intestinal tract, in the absence of dyspeptic symptoms. Prednisone was given without effect. Bilateral broncho-pneumonia, unresponsive to antibiotics, finally developed, and this, coupled with increasingly severe bleeding from the gut and progressive anaemia, proved fatal. Post-mortem examination revealed the typical appearances of chronic lymphocytic leukaemia, with widespread infiltrations of the liver, spleen and lymph glands and evidence of recent bleeding from an area of duodenal ulceration.

The clinical history and chart (Fig. 50) of this patient's progress illustrate the course of an initially florid leukaemia, with generalized lymphadenopathy, under the influence successively of TEM, radiotherapy, and chlorambucil. Much the best haematological response was achieved with chlorambucil, but no form of therapy succeeded in clearing the lymphadenopathy entirely and clinical remissions were only partial. Nevertheless, for nearly 6 years from the time of clinical onset the patient continued at work without severe or disabling symptoms. Post-mortem unexpectedly revealed a bleeding duodenal ulcer, whereas clinically the terminal gastro-intestinal haemorrhage had been thought to be due to thrombocytopenia.

CASE NO. 15 (Fig. 51). Male, aged 63 years, was discovered to have chronic lymphocytic leukaemia during the course of investigations prior to prostatectomy. The haemoglobin was 14·8 gm. per cent, the platelets 300,000 per cu. mm. and the leucocyte count 150,000 per cu. mm., predominantly small lymphocytes. Apart from these blood changes there were no signs or symptoms of leukaemia. No anti-leukaemic treatment was given at this stage, but observation was maintained at monthly intervals. Within 4 months a few

sore throat responded to local antibiotics, and since the patient had then no complaints and the haemoglobin was well maintained, no anti-leukaemic therapy was given, but observation was continued at monthly intervals. After 9 months little change had occurred in the lymphadenopathy, but the haemoglobin level had fallen to 10·6 gm. per cent and the leucocytes had increased to 160,000 per cu. mm. Treatment was therefore initiated with triethylene melamine (TEM), a total of 27·5 mgm. being given over a period of 2 months in the first course. There was general clinical and haematological improvement after this course, the haemoglobin rising to between 13 and 14 gm. per cent, the

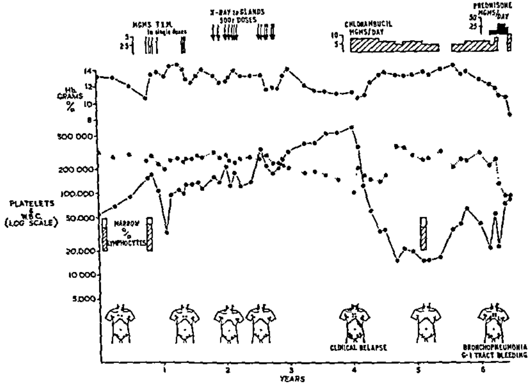

FIG 50

glands becoming a little smaller, and the leucocyte count falling to 33,000 per cu. mm. The haemoglobin response was subsequently maintained, but the leucocyte level rapidly climbed once more and a further course of 15 mgm. TEM was given 4 months later. The response to this second course was poor, and indeed there was a fall in haemoglobin and the lymphadenopathy increased at this time, while the leucocyte level was unaffected. The clinical state was reasonably satisfactory, however, and the patient had few complaints for the next 6 months, when the cervical glands became larger and somewhat painful and recurrent throat infections became troublesome.

Radiotherapy to the enlarged lymph glands was now given, the most prominent groups being treated in turn. A total of about 6,500 r was given intermittently over the next year

of septic lesions of the skin, especially of the forearms and scalp, and although each lesion healed gradually with local antibiotic ointment, fresh lesions persistently appeared. The lesions were not staphylococcal but showed variable microbial flora. Study of the plasma protein electrophoretic pattern revealed no deficiency of gamma-globulins and no other abnormality.

This case report illustrates once more the chance discovery of chronic lymphocytic leukaemia in a patient admitted to hospital for reasons unconnected with leukaemia. It also shows an excellent response of widely generalized disease to chlorambucil and illustrates the need for maintenance rather than intermittent treatment. The low-grade infections of the skin are a typical example of the tendency for patients with this disease to exhibit lowered resistance to infection. In this case the tendency could not be correlated with a deficiency of gamma-globulins.

CASE No. 16 (Fig. 52). Female, aged 71 years, was admitted to hospital in August 1956 with acute diarrhoea, persistent vomiting and nausea. Examination revealed moderately enlarged lymph glands in the axillary and inguinal regions, gross splenomegaly, the organ extending into the left iliac fossa, and enlargement of the liver, with the edge palpable 12 cm. below the right costal margin. A blood count showed haemoglobin 15 gm. per cent, platelets 250,000 per cu. mm. and leucocytes 51,400 per cu. mm., with 96 per cent lymphocytes, mostly mature small cells but some having atypical features. The gastro-intestinal symptoms responded rapidly to chlorpromazine and the patient was discharged without any anti-leukaemic treatment having been given. She was eventually referred for treatment in October 1957, after recurrence of persistent vomiting, now unresponsive to chlorpromazine. At this time the haemoglobin had fallen to 10·7 gm. per cent, and the platelets to 120,000 per cu. mm., while the leucocyte count had risen to 147,000 per cu. mm. She was complaining of nausea and vomiting, dyspnoea on exertion, swelling of the ankles, and dizzy attacks. There was moderate enlargement of lymph nodes at all palpable sites and the spleen was enormously enlarged (see Plate XXI). The liver edge was felt 10 cm. below the costal margin. There was considerable oedema of the ankles, presumed to be due to obstruction to the venous and lymphatic return from the legs, since there was no evidence of cardiac or renal disease. Chest X-rays showed no definite hilar or mediastinal glandular enlargement, but there were irregular densities in the lung fields suggestive of small pulmonary deposits.

Treatment with chlorambucil was initiated in a dose of 10 mgm. daily and continued until the leucocyte count had dropped to 26,000 per cu. mm. There was considerable subjective improvement but only slight regression in the splenomegaly. Maintenance treatment with reduced doses of chlorambucil was necessary since the leucocyte count rose rapidly whenever the drug was stopped. A small increase in haemoglobin level took place but the clinical condition remained stationary, and neither the use of prednisone nor radiotherapy to the spleen effected any significant improvement in the general condition or in the extent of splenomegaly. After a period of 9 months, rapid deterioration occurred, with great increase in lymphadenopathy and rise in leucocyte level, and although the leucocytes were again reduced by intensive chemotherapy, the clinical deterioration was unaffected, bilateral broncho-pneumonia developed and death took place 2 years after the diagnosis had first been made.

cervical glands became palpable and over the next year generalized lymphadenopathy developed, with glands about 1–2 cm. in diameter at all superficial sites. The spleen enlarged to 3 cm. below the left costal margin and the liver edge became palpable. During this time there were also two episodes of otitis externa, each responding satisfactorily to local treatment. Sixteen months after diagnosis had been made there was generalized moderate lymphadenopathy, hepato-splenomegaly, and symptoms of lassitude and malaise. A group of enlarged para-aortic glands could be felt. The haemoglobin level, which had been following a fluctuating but slightly downhill course, had reached 11·4 gm. per cent, while the leucocytes had risen to 270,000 per cu. mm.

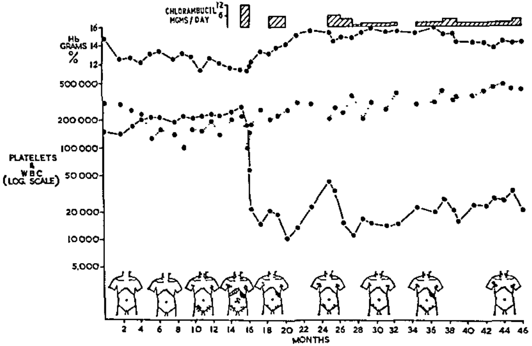

FIG. 51.

Treatment was started with chlorambucil in relatively high dosage of 12 mgm. daily since the patient was able to attend for weekly blood counts. The drug was stopped after 4 weeks, when the leucocyte count had fallen to 56,000 per cu. mm., and the fall continued for a further 3 weeks, reaching a level of 15,000 per cu. mm. At this time there was significant improvement in symptoms and the physical signs had much diminished. Maintenance treatment with chlorambucil proved necessary, since whenever it was stopped recrudescence of lymphadenopathy and splenomegaly soon occurred and the leucocyte count rose rapidly. By varying dosage between 4 mgm. and 2 mgm. daily the leucocytes have been kept between 10,000 and 30,000 per cu. mm. and the patient has been well enough to remain at work and live a normal life.

A troublesome, though minor, complication in the case was the repeated development

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This case-history illustrates the unusual presentation of chronic lymphocytic leukaemia with gross splenomegaly, and the failure of either chemotherapy or radiation to control the progress of the disease, except to a very minor extent, despite reduction in the leucocyte level. The disease was already in a far-advanced state before treatment was begun,

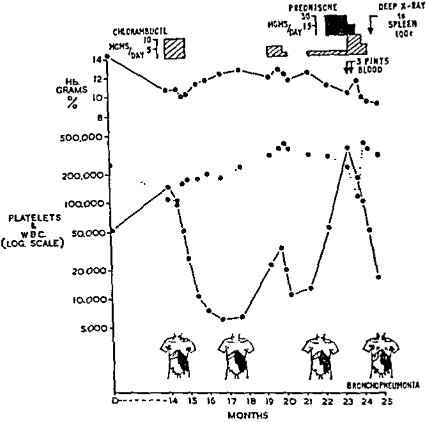

FIG. 52.

and from the huge size of the spleen at first examination it seems likely that the leukaemic process had begun many months earlier.

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females, and that it affects chiefly children and young adults. It has occasionally been observed in patients over 40 years of age and congenital cases have been reported (Morrison *et al.*, 1939).

Symptomatology and associated pathology. The general symptoms are those of acute leukaemia, with rapidly increasing pallor, weakness, loss of weight, bone pains, stomatitis and pharyngitis, and haemorrhagic phenomena. Accompanying this picture and sometimes dominating it, especially in children, are severe symptoms arising from the presence of rapidly growing tumours, often situated in the orbit and causing protrusion of the eyeball, partial or complete blindness and oculomotor paralysis. The skull bones are involved in over 75 per cent of cases. Other common sites of skeletal involvement are the temporal bones, the paranasal sinuses, sternum, ribs, vertebrae, and pelvis. Less often tumours may be conspicuous in the long bones. The chloromatous masses are usually found beneath the periosteum, but they occur also within the marrow cavity eroding both the medullary and cortical bone. Erosions with cortical thinning, localized areas of translucency, periosteal elevation and new bone formation, either parallel with the cortex or at right angles to it, may be seen in radiographs, but, as Kemp and Williams (1941) emphasized, the X-ray changes are not diagnostic of chloroma, being similar to those often observed in non-chloromatous forms of leukaemia in childhood. Predominant involvement of skull and orbits is, however, very suggestive of chloroma. Non-leukaemic conditions, such as congenital syphilitic osteo-periostitis, secondary neuroblastoma, and osteomyelitis, which may be suggested by the radiological appearances, are usually readily distinguished by cytological examination of the blood and bone marrow.

In addition to bone involvement, chloromatous deposits may be found in the lymph glands and viscera, including the kidneys, liver, lungs, pleura, heart, pericardium, intestines and spleen. Indeed, examples of tumour formation in muscles, the breasts, the spinal meninges, and many other sites throughout the body have been reported and no organ or tissue appears exempt, although deposits in the brain and spinal cord are rare. A great variety of symptoms and signs may arise as a result of pressure from chloromatous masses upon local structures, and among these are neurological disturbances such as paraplegia, since although the spinal cord is not directly affected by deposits, pressure upon it may result from the growth of epidural and meningeal tumours (Critchley and Greenfield, 1930).

Nodular tumours may be seen in the bone marrow at autopsy, and elsewhere the marrow sometimes manifests a greenish colour, but more often it is creamy grey or pink, soft, and not unlike the marrow in typical acute leukaemia.

When tumours from any site are sectioned they appear homogeneous and vary in colour from pale yellowish-green to deep grass-green. The green colour fades gradually on exposure to air but can be preserved by mounting in glycerine. The nature of the green pigment remains uncertain, possible theories being that it is a protoporphyrin (Dustin and Thomas, 1938) or a choleglobin derivative (Humble, 1946). Microscopically the tumour masses are seen to be composed of primitive cells of myeloblastic or myelomonocytic type, with little supporting stroma. They show signs of local invasiveness and are more like malignant metastases than are typical leukaemic infiltrates.

Blood changes. The blood picture is indistinguishable from that of acute myeloblastic or myelomonocytic leukaemia, although the peripheral leucocyte count is not often greatly

CHAPTER 15

UNUSUAL VARIETIES OF LEUKAEMIA

MANY of the less common modes of presentation, system involvements, and haematological features of the major varieties of leukaemia have been described in the preceding chapters. Some of these are strikingly atypical, as when chronic lymphocytic leukaemia first manifests itself as leukaemic erythrodermia or Mickulicz's syndrome, or when acute leukaemia is fully aleukaemic in the peripheral blood or initially pancytopenic with an aplastic marrow, but they are all eventually recognizable as variants of one or other of the common forms of leukaemia.

In the present chapter a rather different group of diseases is to be discussed. These are all more or less clearly acceptable as members of the general leukaemia class, but they have been held to differ so substantially from the usual forms of leukaemia that separate consideration is called for. Among them are chloroma, erythraemic myelosis, chronic neutrophilic, eosinophilic, basophilic and mast cell leukaemias, megakaryocytic and plasma cell leukaemias, leukosarcoma and chronic monocytic leukaemia.

Chloroma

Chloroma or chloroleukaemia is a condition in which the general symptoms and signs of acute leukaemia are found in association with localized tumour formation, the tumour masses being green in colour and occurring principally in relation to the periosteum and bones of the skull, although almost any part of the body may be affected.

The very characteristic and striking appearance of the green tumours had been observed and reported early in the nineteenth century, but the disease was not clearly recognized to be a variety of leukaemia until careful studies of the blood were made by Waldstein (1883), Von Recklinghausen (1885) and Dock (1893). Forkner (1938) surveyed the earlier literature on chloroma and showed how the original belief that the tumours were of lymphoid origin gave place to the concept that they were part of the pathological process in an unusual form of acute or subacute myeloblastic leukaemia. This concept is now generally accepted and the cytology of the chloromatous masses and that of the leucocyte precursors in the blood and bone marrow are usually thought to be myeloblastic although occasional examples of monocytic or myelo-monocytic chloroma have been reported (Ross, 1955).

The condition is uncommon, probably more so at present than in the past. Edgerton (1947) found reports of 336 cases previously published, but in many of these the diagnosis was doubtful. In recent years some five or six case reports of chloroma have been published annually. Study of these isolated reports and of the several collected reviews, such as those of Burgess (1912), Brannan (1926), Kandel (1937), Atkinson (1939) and Kemp and Williams (1941), shows that the disease is about twice as common in males as in

females, and that it affects chiefly children and young adults. It has occasionally been observed in patients over 40 years of age and congenital cases have been reported (Morrison *et al.*, 1939).

Symptomatology and associated pathology. The general symptoms are those of acute leukaemia, with rapidly increasing pallor, weakness, loss of weight, bone pains, stomatitis and pharyngitis, and haemorrhagic phenomena. Accompanying this picture and sometimes dominating it, especially in children, are severe symptoms arising from the presence of rapidly growing tumours, often situated in the orbit and causing protrusion of the eyeball, partial or complete blindness and oculomotor paralysis. The skull bones are involved in over 75 per cent of cases. Other common sites of skeletal involvement are the temporal bones, the paranasal sinuses, sternum, ribs, vertebrae, and pelvis. Less often tumours may be conspicuous in the long bones. The chloromatous masses are usually found beneath the periosteum, but they occur also within the marrow cavity eroding both the medullary and cortical bone. Erosions with cortical thinning, localized areas of translucency, periosteal elevation and new bone formation, either parallel with the cortex or at right angles to it, may be seen in radiographs, but, as Kemp and Williams (1941) emphasized, the X-ray changes are not diagnostic of chloroma, being similar to those often observed in non-chloromatous forms of leukaemia in childhood. Predominant involvement of skull and orbits is, however, very suggestive of chloroma. Non-leukaemic conditions, such as congenital syphilitic osteo-periostitis, secondary neuroblastoma, and osteomyelitis, which may be suggested by the radiological appearances, are usually readily distinguished by cytological examination of the blood and bone marrow.

In addition to bone involvement, chloromatous deposits may be found in the lymph glands and viscera, including the kidneys, liver, lungs, pleura, heart, pericardium, intestines and spleen. Indeed, examples of tumour formation in muscles, the breasts, the spinal meninges, and many other sites throughout the body have been reported and no organ or tissue appears exempt, although deposits in the brain and spinal cord are rare. A great variety of symptoms and signs may arise as a result of pressure from chloromatous masses upon local structures, and among these are neurological disturbances such as paraplegia, since although the spinal cord is not directly affected by deposits, pressure upon it may result from the growth of epidural and meningeal tumours (Critchley and Greenfield, 1930).

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Blood changes. The blood picture is indistinguishable from that of acute myeloblastic or myelomonocytic leukaemia, although the peripheral leucocyte count is not often greatly

raised and an aleukaemic phase may persist for some days or weeks after local tumour formation has first been noted. A frankly leukaemic picture, with a high percentage of primitive cells in the blood, eventually develops in almost every case. During aleukaemic phases the diagnosis can, however, usually be established with certainty by bone-marrow biopsy, when the appearances of massive leukaemic infiltration will be found.

Treatment. The same forms of treatment are applicable as used in non-chloromatous acute myeloblastic leukaemia. In addition to the usual antimetabolite chemotherapy and supportive treatment with transfusions and antibiotics when appropriate, cautious irradiation of chloroma masses causing pressure effects is advisable. The course of the disease, the likelihood of inducing remissions with anti-leukaemic therapy, and the eventual outcome are similar to those of acute leukaemia in general, and it is unusual for patients to survive more than 8 months to a year from the time of diagnosis even when temporary remissions have been successfully induced.

Erythraemic Myelosis and Erythroleukaemia

In an extensive series of publications since 1917, Di Guglielmo has developed, with illustrative case reports, the concept that erythroblastic tissue could give rise to a malignant proliferative blood disease essentially similar to the leukaemic proliferation arising from leucocyte precursors (Di Guglielmo, 1917, 1923, 1946, 1956). The emergence of a clear-cut clinical and haematological picture of "erythraemic myelosis", as this malignant erythroblastic hyperplasia has been called, has long been hampered by the rarity of examples of the disease and the frequent occurrence of exaggerated erythroblastic hyperplasia in the bone marrow and erythroblastosis in the peripheral blood in states of non-malignant increase in erythropoietic activity, as in haemolytic anaemias of many kinds. Thalassaemia major proved especially confusing in this respect and more than half the cases reported as erythraemic myelosis, chiefly by Italian authors, between the years 1924 and 1940, appear in retrospect to be examples of thalassaemia or haemolytic disease of the newborn (Moeschlin, 1940, 1951).

Another source of confusion is the existence of erythroblastic hyperplasia simultaneously with acute leukaemic, especially myeloblastic, proliferation. An occasional erythroblast is to be found in the peripheral blood at some stage in most cases of acute leukaemia, and red cell precursors may sometimes be numerous, perhaps more numerous than leucoblasts. When erythroblasts are present in substantial proportion, the mixed proliferation may be referred to as "erythroleukaemia", and some writers have expressed the view that most, if not all, cases of supposedly pure erythraemic myelosis are in reality erythroleukaemic, with mixed myeloblastic and erythroblastic hyperplasia, and that the longer the patient lives, the more likely is the apparent erythraemic myelosis to become an obvious erythroleukaemia or a frank acute leukaemia (Martin and Bayrd, 1954; Whitby and Britton, 1957; Dameshek and Baldini, 1958). Studies of the glycogen content of erythroblasts may be helpful in clarifying this issue, since the P.A.S. reaction has been found to be negative in erythroblasts from most cases of acute leukaemia but strongly positive in those from erythraemic myelosis and from erythroleukaemia with a conspicuous erythraemic component (Quaglino and Hayhoe, 1959). Transitions undoubtedly take place, as they do between other leukaemic and paraleukaemic disorders, such as

polycythaemia vera and chronic myeloid leukaemia (see Chapter 16), and, indeed, few haematologists would claim strict pathological autonomy for any member of the myeloproliferative or lymphoproliferative diseases, but enough cases of erythraemic myelosis in pure form have now been observed for the disease to be accepted as a recognizable, if not sharply separated, entity. References to many of the published case reports have been given by Britton and Neumark (1949), Schwartz and Critchlow (1952) and by Haranghy, Doczi, Székely and Spielmann (1958), whose monograph on leukaemic erythromyelosis deals fully with the clinical and pathological aspects of this group of diseases.

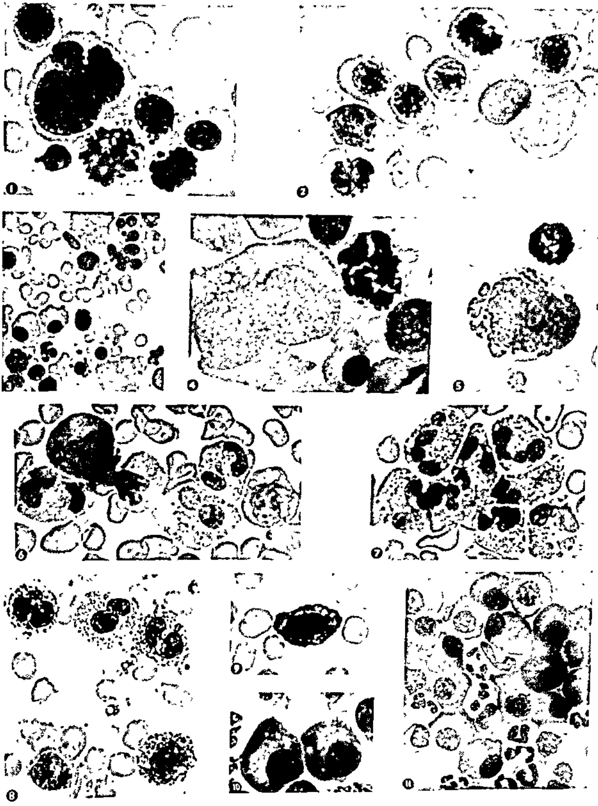
Acute erythraemic myelosis. The characteristic features of this disease as described by Di Guglielmo include rapid onset with severe anaemia, irregular remittent fever, marked splenomegaly and slight hepatomegaly, and an acute course of one or two months to an invariably fatal termination. The blood changes are dramatic, with severe anaemia and many circulating erythroblasts of all stages of maturity, ranging in number from 3,000 to 300,000 per cu. mm. The earlier red cell precursors, proerythroblasts and basophilic erythroblasts, usually predominate, and many show atypical cytological features, including multiple nuclei and lack of synchronism between nuclear and cytoplasmic maturation. The cells may be chiefly normoblastic, macronormoblastic, or megaloblastic, and mixed appearances are common. The relatively low proportion or complete absence of orthochromic normoblasts shows a parallel with the absence of intermediate granulocyte precursors in acute myeloblastic leukaemia and has been referred to as the "hiatus erythraemicus" (Baserga, 1938). The mature red cells show much variability in appearance from case to case, being sometimes normochromic and normocytic, but more often macrocytic with anisocytosis and poikilocytosis. Reticulocytosis is not usually prominent, in contrast with the findings in haemolytic anaemia, and the presence of peripheral erythroblastosis without reticulocytosis is a helpful pointer to the diagnosis. Leucocytes may be decreased, normal or increased in numbers, but are rarely more than 30,000 per cu. mm. A shift to the left, with occasional metamyelocytes and myelocytes, may be seen, but in the classical form of the disease few or no immature leucocyte precursors are to be found. Thrombocytopenia is commonly severe and, clinically, haemorrhagic manifestations resemble those of acute leukaemia.

The bone marrow shows remarkable erythroblastic hyperplasia, with great preponderance of early basophilic cells and considerable numbers of proerythroblasts, haemocytoblasts and primitive reticulum cells. Leucopoietic and thrombopoietic cells are much reduced in numbers. Mitotic and other cytological irregularities are frequent among the erythroblasts (Plate XXVIII, Figs. 1 to 5), and megaloblastoid forms may be conspicuous. Nevertheless, the concentration of vitamin B_{12} in the serum has been reported to be normal and there is no response to parenteral administration of vitamin B_{12} (Dameshek and Baldini, 1958).

Post-mortem examination reveals diffuse hyperplasia of early erythroblasts throughout the haemopoietic system and extensive, though variable, infiltration in the liver, spleen, lymph glands, and often in other viscera. Haemorrhagic lesions may be conspicuous.

The pure form of acute erythraemic myelosis differs clinically from acute leukaemia in the greater extent of splenomegaly, the general absence of significant lymph-gland enlargement and the rarity of ulcerating and necrotic oral lesions. Haematologically the differential diagnosis from haemolytic anaemia, especially thalassaemia, may be difficult,

PLATE XXVIII



been reluctant to diagnose erythroleukaemia unless the numbers of erythroblasts in both the blood and bone marrow approach or exceed those of the leucocytes. This is really a question of semantics, of no great clinical or haematological importance provided that the possible concurrent existence of widely variable degrees of erythraemic and leukaemic proliferation is clearly appreciated.

The leukaemic component of erythroleukaemia is usually myeloblastic, but may be myelomonocytic or monocytic (Hindmarsh and Wickham, 1955). Lymphoblastic leukaemia does not appear to share the association, presumably because lymphopoiesis differs in site and nature from medullary erythropoiesis and granulopoiesis.

Erythroleukaemia pursues a course like that of acute myeloblastic leukaemia, with similar signs and symptoms, and a comparable prognosis. There is severe anaemia, moderate or slight hepato-splenomegaly, fever, stomatitis, haemorrhagic phenomena and a tendency to succumb to intercurrent infection. The blood, the bone marrow, and, at post-mortem examination, many of the organs and tissues, show extensive infiltrations with erythroblasts as well as with leucoblasts.

Treatment. This group of diseases has not so far proved amenable to treatment. Temporary benefit may be derived from fresh blood transfusion, and definite remission of the disease has sometimes followed upon this measure (Mackenzie and Stephenson, 1952). Splenectomy has been tried in a number of cases, with little improvement, and, indeed, a sharp rise in the number of peripheral erythroblasts has been observed after the operation. The use of antimetabolites may lead to remission, especially in erythroleukaemias with a strong leukaemic component, but predominantly erythraemic cases seem usually to have been refractory, although the literature contains few reports of attempted chemotherapy. It would certainly be reasonable to employ much the same methods in treating acute erythraemic myelosis and erythroleukaemia as are used in myeloblastic and myelo-monocytic leukaemias, including trials with antimetabolites and adrenocortical hormones, and supportive therapy with transfusions and antibiotics as required. In chronic erythraemic myelosis there is less urgent need for aggressive chemotherapy, but cautious use of purine antagonists, busulphan, or radioactive phosphorus would be worth trying in the hope of reducing abnormal erythropoiesis to a point where a return to normal might become possible, comparable with the emergence of normal leucopoiesis when chemotherapy effectively reduces the pathological leucopoiesis of leukaemia.

Variants of Chronic Granulocytic Leukaemia

In chronic granulocytic leukaemia very wide variations occur in the differential leucocyte count of the peripheral blood. Characteristically, as we have seen earlier, there are large numbers of granulocyte precursors as well as mature neutrophils, and increased proportions of eosinophils and basophils are present. Monocytes, too, are often greatly increased both absolutely and relatively. Among the more remarkable variations from this pattern of general granulocytic and monocytic increase are those in which mature neutrophils, eosinophils (Plate XXVIII, Figs. 6 and 7) or basophils (Plate XXVIII, Fig. 8) respectively predominate in the peripheral blood. Such cases are extremely rare, but several in which adequate investigation has suggested the leukaemic nature of the disease have been described under the names neutrophilic, eosinophilic and basophilic leukaemia.

but clinically erythraemic myelosis has no family incidence, runs a much more acute and severe course, and does not show the characteristic bone changes seen in thalassaemia.

Leucoblastic proliferation may remain absent throughout the whole course of the disease but transition to erythroleukaemia, with the appearance of myeloblasts in the bone marrow and peripheral blood, not uncommonly takes place.

Chronic erythraemic myelosis. This disorder is related to acute erythraemic myelosis much as chronic leukaemias are related to acute. Most cases have been described in adults, although Di Guglielmo and Quattrin (1942) observed a typical example in a girl aged 12 years. The clinical course is more prolonged than that of the acute form and the signs and symptoms develop more slowly, but again include splenomegaly, hepatomegaly, anaemia and fever. Thrombocytopenia is less marked. The disease usually proves fatal after 1 to 2 years, although it may continue to develop slowly for as long as 10 years (Dameshek and Baldini, 1958). The peripheral erythroblastosis ranges from 5,000 to 100,000 cells per cu. mm. and these are mostly late normoblasts, with smaller numbers of intermediate erythroblasts and few basophilic precursors. Erythroid hyperplasia preponderates in the bone marrow, with myeloid-erythroid ratio as low as 1 to 30 (Heilmeyer and Schöner, 1941), the majority of the erythroblasts being at intermediate or late stages of maturity, although primitive forms also show a substantial increase. There is hypoplasia of both leucopoietic and thrombopoietic tissue. Mitotic and other cytological aberrations in the nucleated red cell precursors are present but less conspicuous than in acute erythraemic myelosis, while an increase in reticulocytes is more common.

At autopsy, generalized infiltration of haemopoietic organs and viscera with erythroblasts is found, comparable to the organ infiltrations with leucocytes in chronic leukaemias.

Cases intermediate between acute and chronic erythraemic myelosis, both in clinical severity and in the degree of erythroblastic immaturity, have been reported, and may be regarded as subacute forms.

Erythroleukaemia. More common than the pure forms of erythraemic myelosis is a *mixed erythraemic and leukaemic proliferation.* This condition, erythroleukaemia, is not clearly defined, since it may range haematologically from an almost pure erythraemic myelosis with only a few myeloblasts and promyelocytes to an equally pure acute leukaemia with minimal peripheral erythroblastosis and only slight increase in early erythroblasts in the marrow. Since it is not easy to distinguish erythraemic proliferation from normal erythropoietic activity in a leukaemic marrow, and since orthochromatic normoblasts frequently appear in the peripheral blood in acute leukaemia as a temporary phenomenon especially during the early phases of remission, most haematologists have

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PLATE XXVIII

ATYPICAL FORMS OF LEUKAEMIA AND RELATED DISEASES

1, 2, 3, 4, 5. Atypical erythroblasts in erythraemic myelosis. (3 × 600, remainder × 1000)

6 and 7. Vacuolated and irregularly granular eosinophils in diffuse eosinophilic collagen disease. (× 1000)

8. Intense basophilia in chronic granulocytic leukaemia. (× 1000)

9. A tissue mast cell. (× 1000)

10 and 11. Plasma cells in a marrow smear from multiple myelomatosis. (× 1000, × 600)

been reluctant to diagnose erythroleukaemia unless the numbers of erythroblasts in both the blood and bone marrow approach or exceed those of the leucocytes. This is really a question of semantics, of no great clinical or haematological importance provided that the possible concurrent existence of widely variable degrees of erythraemic and leukaemic proliferation is clearly appreciated.

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Neutrophilic leukaemia. Rathery (1902) first reported an example of this unusual form of leukaemia. His patient had the clinical picture of chronic granulocytic leukaemia, with moderate anaemia and gross splenomegaly, and the peripheral leucocyte count was 41,000 per cu. mm. Mature polymorphonuclear neutrophils made up 79 per cent of the count, there were 19 per cent monocytes, and only 1·3 per cent myelocytes. A similar case was described by Hirschfeld (1914). Both these patients died following splenectomy, and at autopsy the changes of chronic granulocytic leukaemia were found in their organs. Several further case reports, such as those of Tuohy (1920), Naegeli (1931) and Émile-Weil and Sée (1932), confirmed the existence of this variant of leukaemia and suggested that its evolution might be somewhat slower and the course more benign than that of typical chronic granulocytic leukaemia. Whitby and Britton (1957) drew attention to the difficulty of distinguishing between leukaemic and leukaemoid polymorphonuclear reactions, and implied that most reported cases were not truly leukaemic; this interpretation does not seem justified. As Forkner (1938) pointed out, those cases which had been studied *post mortem* showed proliferation and infiltration involving both immature and mature granulocytes in the haemopoietic organs and elsewhere identical with the usual picture of chronic granulocytic leukaemia. The difference between neutrophilic leukaemia and the ordinary granulocytic form would therefore appear to be one concerning the mechanism of release of cells into the circulation rather than an essential difference in the cell type undergoing leukaemic transformation. Studies of leucocyte alkaline phosphatase activity might be expected to be valuable in assisting the diagnosis of neutrophilic leukaemia, since the enzyme levels should be severely depressed by analogy with those in chronic granulocytic leukaemia.

Eosinophilic leukaemia. Since the early observations of Stillman (1912), Schmidt-Weyland (1925) and Hay and Evans (1929) much has been written about the existence and characteristics of eosinophilic leukaemia. This title might reasonably be given to a disorder having the general clinical and haematological features of granulocytic leukaemia but an overwhelming preponderance of eosinophils and eosinophil precursors in the blood and bone marrow throughout the course of the disease until death or terminal myeloblastic transformation. Unequivocal examples of such a disorder are extremely rare, if, indeed, they exist at all, and the great majority of cases reported as eosinophilic leukaemia appear to be leukaemoid reactions or transitory phases of eosinophilia during the course of otherwise typical acute or chronic granulocytic leukaemia.

The relationship of eosinophilia and leukaemia has been carefully analysed by Bousser (1957), who collected data from the literature and from colleagues on all suspected cases of eosinophilic leukaemia in which adequate clinical and haematological investigations had been performed. The chief conclusions from this most interesting and informative analysis are as follows.

1. Marked eosinophilia may develop during the course of chronic granulocytic leukaemia and temporarily dominate the blood and marrow pictures, and a similar temporary eosinophilia, often with cytologically abnormal eosinophils, may occur in acute and sub-acute myeloblastic leukaemias. This phenomenon is a transient one and gives place to the usual haematological pattern after a variable period of time. It is possible that parasitic infestation or allergic disorder may predispose to its development in some cases (Tolentino, 1948). Cases falling into this general category include

those of Astaldi and Curti (1947), Tolentino (1948), Muller *et al.* (1949) and Ravetta (1949, 1952).

2. Most of the remaining cases reported as eosinophilic leukaemia appear to be leukaemoid reactions to a generalized disorder with variable clinical features designated by Engfeldt and Zetterström (1956) as "disseminated eosinophilic collagen disease". The pathological features of this disorder consist of necrotic, oedematous and granulomatous lesions of the interstitial connective tissue, with variable arterial lesions, tissue eosinophilia and increase in eosinophils in the peripheral blood. While blood eosinophilia is a constant feature of the disease, only occasionally do patients develop so high and persistent an eosinophilia as to suggest leukaemia. The clinical picture is extremely polymorphous, since the lesions of interstitial tissue may occur predominantly in many possible sites, including the lungs and pleura, with asthmatic attacks, fluctuating pulmonary infiltrates and pleural effusions; the heart, with pericarditis, endocarditis, and congestive failure; the kidneys, with albuminuria, haematuria and arterial hypertension; and the skin, with pruritus, urticaria, oedema, erythematous, papular or pustular rashes, cutaneous or subcutaneous nodules and purpura. In addition there may be enlargement of liver, spleen and lymph glands, pains in muscles and joints, wasting and intermittent fever. The diagnosis is suggested by the presence of some combination of these system involvements and the existence of eosinophilia. Within the general heading "disseminated eosinophilic collagen disease" may be included such clinically diverse but pathologically related states as parietal fibroplastic endocarditis (Loeffler, 1952), eosinophil myocarditis, periarteritis nodosa and asthma with eosinophilia (Rackemann and Greene, 1939), allergic granulomatosis and angiitis (Churg and Strauss, 1951) and the syndrome of disseminated visceral lesions with extreme eosinophilia observed in young children by Zuelzer and Apt (1949).

From his study of the clinical and pathological records of 29 adequately documented cases reported as eosinophilic leukaemia, Bousser concluded that all 16 in which there was no terminal myeloblastic transformation were in reality examples of disseminated eosinophilic collagen disease. Of the 13 cases showing a terminal myeloblastic change, 7 had manifested characteristic features of the same disease, with myocardial, pulmonary or dermatological lesions, while the remaining 6 were probably cases of transient eosinophilia during the course of acute or chronic granulocytic leukaemia.

The clinical pattern of most reported cases clearly conforms with that of the disseminated allergic disease, and they may reasonably be regarded as illustrating an extreme form of eosinophilic leukaemoid reaction, rather than a true leukaemia. This interpretation is further reinforced by the haematological observation that in most cases the eosinophils have been predominantly mature cells, and when a terminal myeloblastosis occurred there has often been a remarkably complete absence of intermediate cells, myelocytes and metamyelocytes, between the mature eosinophils and the undifferentiated precursor cells. This hiatus differs from the ordinary "hiatus leukaemicus" of acute leukaemia in that the proportion of mature cells has generally been vastly greater in relation to the primitive cells than is the case in acute leukaemia. Another feature against a leukaemic interpretation is the lack of correlation between the nature and extent of the eosinophilia, including especially the proportion of precursor cells, and the clinical severity of the disease. Eosinophilias with predominantly mature cells, and with counts ranging from 20,000 to 250,000 cells per cu. mm., have been observed in cases with severe and short courses of a few days

or weeks, but also in more chronic cases, surviving as long as 4 or 5 years. The clinical subdivision into acute and chronic cases does not seem to be paralleled by an associated haematological differentiation, of the kind seen in acute and chronic leukaemias.

As far as treatment is concerned, the only cases suitable for radiotherapy or chemotherapy along anti-leukaemic lines are those where eosinophilia is clearly part of an acute or chronic granulocytic leukaemia, and here orthodox therapeutic measures are applicable.

When a considerable eosinophilia is found with predominantly mature cells, and even when there are also precursor eosinophils and myeloblasts present, but no general neutrophil granulocytic increase, a careful search should be made for possible causes of eosinophilic leukaemoid reaction, including parasitic infestation, cancer, Hodgkin's disease, and especially the syndrome of disseminated collagen disease. If evidence is found to support the last possibility, treatment with corticosteroids may be temporarily effective and there is a case for giving anticoagulants to reduce the risk of thrombosis and ischaemic necrosis resulting from arterial lesions (Gerbaux *et al.*, 1956).

Basophilic leukaemia. An increase in basophil leucocytes both relatively and absolutely is an extremely common feature of chronic granulocytic leukaemia, and it is not at all unusual to find these cells constituting 20 to 30 per cent of the peripheral leucocyte count, regardless of its total height. These basophils are frequently smaller in size and have fewer granules than do the basophils in a normal blood-smear, but they are clearly recognizable and provide a most characteristic component of the blood picture in chronic granulocytic leukaemia. When the basophil count has been inordinately high, making up from 50 to 80 per cent of the total leucocyte count, the designation "basophilic" or "mast leucocyte" leukaemia has sometimes been applied. Forkner (1938) found rather meagre data in the medical literature referring to 11 such cases, but concluded that most if not all of these represented merely an exaggeration of the basophilia of chronic granulocytic leukaemia. The question was further discussed by Doan and Reinhart (1941), who found 5 possible cases among 300 with granulocytic leukaemia, but in more recent years the diagnosis of basophilic leukaemia has very rarely been made. Nevertheless, occasional reports have continued to draw attention to the occurrence of very high basophilia, usually in otherwise typical chronic granulocytic leukaemia (Casey, Nettles and Hidden, 1946; Lennert, Köster and Martin, 1956). A more remarkable phenomenon, described by Hule (1950), was the presence of 28 to 35 per cent of finely or coarsely granular basophils in the peripheral blood and 90 per cent of immature basophils in the marrow of a patient whose clinical course was like that of acute leukaemia. It remains somewhat doubtful, however, whether a separable leukaemia of the basophils exists.

Tissue Mast Cell Leukaemia

Morphologically, tissue mast cells are clearly distinguishable in smears of blood or marrow from basophil leucocytes. They have a small round nucleus and a relatively large amount of cytoplasm, usually densely packed with basophilic granules (Plate XXVIII, Fig. 9). The precursors of the blood basophil have a nucleus almost filling the cell and a light scattering of basophil granules over nucleus and cytoplasm alike, while mature basophils have an irregular polymorphous nucleus quite unlike that of the tissue mast cell. This distinction is important since most cases reported as "mast cell" leukaemia

refer to the "mast leucocyte" or basophil polymorphonuclear cell and not to the tissue mast cell.

Only two cases of tissue mast cell leukaemia, proper, have hitherto been reported (Efrati, Klajman and Spitz, 1957; Friedman *et al.*, 1958), but their relation on the one hand to chronic granulocytic leukaemia and on the other to the skin disease, urticaria pigmentosa, deserves brief consideration.

In chronic granulocytic leukaemia tissue mast cells, unlike basophil leucocytes, are not significantly increased in haemopoietic sites or elsewhere, and do not appear in the peripheral blood. In the case reported by Efrati and his colleagues there was scarcely any increase in granular leucocytes or their precursors in the blood, even when the total nucleated cell count rose to 62,000 per cu. mm. At that stage there were 50,360 tissue mast cells per cu. mm. and only 6,200 segmented neutrophils, 620 stab cells and 207 neutrophil myelocytes per cu. mm. There was an eosinophilia, with 3,100 cells per cu. mm., and marked increase in eosinophils was also found in the spleen and bone marrow at autopsy, but this finding of parallel eosinophilia and mast cell increase has been observed in many mast cell disorders and appears more likely to be related to complementary functions of these two cell types in histamine release and disposal (Riley, 1954), than to dual involvement in a neoplastic or leukaemic process. In the case described by Friedman and his associates, eosinophilia was not observed, and again there was very little leucocytosis of normal granular cells, even when the peripheral nucleated cell count rose to 100,000 per cu. mm. Neither examination of the bone marrow nor histological studies of tissues from many sites *post mortem* suggested myeloid proliferation or infiltration and the mast cell form of leukaemia seems clearly separate from the chronic granulocytic form.

A great increase in tissue mast cells has long been known to occur in the skin in urticaria pigmentosa, and in recent years there has been considerable interest in the extension of mast cell proliferation to organs other than the skin. Ellis (1949) found numerous mast cells in the lymph nodes, bone marrow and other organs of an infant with congenital urticaria pigmentosa, and Sagher, Cohen and Schor (1952) described infiltration of the bones in another case. A radiological survey of 45 patients with urticaria pigmentosa disclosed bone lesions in 15, while increased numbers of mast cells have often been found in bone-marrow smears from patients with this skin disease (Sagher, 1956). Invasion of the peripheral blood by tissue mast cells was first reported by Hissard, Moncourier and Jacquot (1951). Their patient showed skin lesions and mast cell infiltrations in the skin, bone marrow and spleen, and although the disorder was regarded as a systemic mastocytosis of malignant character, the term leukaemia might well have been applied. The same is true of a case reported by Brodeur and Gardner (1956), where an erythematous and urticarial skin rash in a 5-year-old boy was associated with hepato-splenomegaly and mast cell infiltration of the bone marrow, lymph nodes and peripheral blood.

A range of mast cell disorders therefore appears to exist, from the purely cutaneous and benign urticaria pigmentosa through various degrees of systemic mastocytosis to the highly malignant picture of the "leukaemic" cases described by Efrati and Friedman and their associates. The term leukaemia may reasonably be applied to these cases since the clinical course resembles that of leukaemia, with hepato-splenomegaly, anaemia and thrombocytopenia, and the proliferation of mast cells, which are normally present in small numbers in the bone marrow, involves the haemopoietic organs and peripheral blood, and includes

the appearance of mast cell precursors. Both these leukaemic patients showed variable skin lesions, but in neither was the picture that of urticaria pigmentosa, and mast cell infiltration in the skin was absent in one case and slight in the other.

A further remarkable example of systemic mastocytosis perhaps related to mast cell leukaemia, but without infiltration of the bone marrow or invasion of the peripheral blood, was reported by Ende and Cherniss (1958). Their patient, a 35-year-old man, was investigated for episodic flushing and vomiting and a haemorrhagic tendency. There was marked splenomegaly, moderate hepatomegaly, and signs of hypersplenic pancytopenia. There was no skin rash. Splenectomy led to the discovery of extensive mast cell infiltration of the spleen, and the authors suggested that the intermittent flushing attacks might have been due to periodic histamine release from the mast cells. The patient remained well and symptom-free, 9 months after splenectomy, at the time the report was written. In this case, then, the mast cell proliferation appears to have been confined substantially to the spleen, although the hepatic enlargement suggests that some degree of dissemination must have taken place.

Megakaryocytic Leukaemia

Megakaryocytic hyperplasia, often with a great increase in circulating platelets and the presence of megakaryocytes or megakaryocyte fragments in the peripheral blood and even infiltrating organs such as the spleen, liver and lungs, may be found in almost any of the myeloproliferative diseases, including chronic granulocytic leukaemia, polycythaemia vera, and myelofibrosis (Minot and Buckman, 1925; Whitby, 1948). At times the hyperplasia of megakaryocytes and the platelet increase dominates the haematological picture and the term megakaryocytic leukaemia or myelosis may then appear appropriate, but such phases are generally temporary and conversion to one of the major myeloproliferative diseases soon takes place (see also Chapter 16).

Under the term "aleukaemic megakaryocytic myelosis", Favre, Croizat and Guichard (1934) separated a group of cases showing marked enlargement of the spleen and liver, a slowly progressive anaemia, a normal or leuco-erythroblastic nucleated cell picture in the peripheral blood, and extensive megakaryocytic infiltration of the marrow, liver, spleen and other organs. Similar syndromes have very often been reported under such names as "myeloid megakaryocytic hepato-splenomegaly" (Downey and Norland, 1939) and "chronic non-leukaemic myelosis" (Carpenter and Flory, 1941), and in some of these cases megakaryocyte fragments have been numerous in the peripheral blood. The majority of such reports probably referred to myelofibrosis, since in many cases of this disease sections of the bone marrow reveal alternating areas of fibrosis and megakaryocytic hyperplasia. Nevertheless, there is an undoubted place for the term megakaryocytic myelosis to represent a rather ill-defined stage, of short or long duration, in which gross hyperplasia of the megakaryocytes precedes the emergence of a more clearly defined myeloproliferative state, especially myelofibrosis.

A second, much rarer, disorder presenting some parallels with leukaemic processes is haemorrhagic thrombocythaemia. Cases have been described and the literature reviewed by Rowlands and Vaisey (1938), Reid (1940), Mortensen (1948), Spangberg and Zettergren (1949), Fanger, Cella and Lichtman (1954), Hardisty and Wolff (1955) and Woodrow and

Cope (1955). The disorder is characterized by venous thromboses and haemorrhages from mucous membranes, and death may result from cerebral thrombosis or haemorrhage or from severe bleeding from the gastro-intestinal tract. If there is much blood loss a hypochromic microcytic anaemia may be found, but otherwise anaemia is not a prominent feature, and indeed some cases have been definitely polycythaemic. Splenomegaly, moderate in degree, is usual, but lymphadenopathy is not conspicuous. A marked polymorphonuclear leucocytosis commonly occurs, with total counts from 20,000 to 100,000 per cu. mm., chiefly mature cells, and there may be leuco-erythroblastosis. The blood platelets are enormously increased in numbers, sometimes reaching a total of 4 or 5 million per cu. mm., and showing bizarre variations in size and shape. They may be qualitatively defective in the thromboplastin generation test, and this deficiency presumably accounts for the bleeding tendency, although the coagulation time of whole blood remains within normal limits and the bleeding time is little prolonged. The bone marrow shows very numerous and atypical megakaryocytes and at post-mortem examination megakaryocytes or fragments are found in various organs. Proliferation of granulocyte precursors is not excessive and there is no suggestion of chronic granulocytic leukaemia. The disorder clearly falls within the group of myeloproliferative diseases, and is perhaps most closely related to polycythaemia vera.

Mention should finally be made of the so-called acute megakaryoblastic leukaemia of Boros and Korenyi (1931). This case appears in retrospect to be one of acute myelomonocytic leukaemia and no examples of megakaryoblastic leukaemia have subsequently been reported.

Plasma Cell Leukaemia

A few abnormal plasma cells (Plate XXVIII, Figs. 10 and 11) commonly appear in the peripheral blood at some stage during the course of multiple myeloma, and occasionally peripheral plasmacytosis may be a constant and conspicuous feature in individual cases. It is to such cases that the term plasma cell leukaemia has sometimes been applied. The reports of Osgood and Hunter (1934), Patek and Castle (1936), Reiter and Freeman (1937), Newman *et al.* (1952) and Brücher and Weicker (1955) refer to patients having a clinical course like that of acute or subacute leukaemia, with progressive anaemia, and leucocyte counts ranging from 10,000 to 90,000 per cu. mm., with plasma cells invariably present and sometimes overwhelmingly predominant. At autopsy plasma cell infiltration was usually conspicuous in the spleen, liver, and lymph glands, as well as in the bone marrow.

There seems little real justification for separating these cases from multiple myeloma; they show the same type of abnormal plasma cell morphologically, usually have a similar hyperglobulinaemia, and may show Bence-Jones proteinuria. Their clinical course certainly tends to be more acute than the average for multiple myeloma, but this is to be expected of a rapidly disseminating variety of the disease. Whitby and Britton (1957) recognize five chief clinical types of multiple myeloma, those with diffuse or patchy marrow infiltration with plasma cells but no blood changes, those with multiple destructive bone tumours but no blood invasion, those with tumours in bone and elsewhere and with a few or many plasma cells in the circulation, those with minimal bone destruction but a high percentage of peripheral plasma cells, and finally those with extensive infiltration of the marrow and the viscera and large numbers of plasma cells in the blood.

It is clearly not reasonable to separate one or other of these closely related variants as specifically leukaemic; there is no fundamental difference between them.

Lymphosarcoma Cell Leukaemia (Leukosarcoma)

Sternberg (1915) applied the term leukosarcoma to a condition in which an infiltrating lymphosarcoma was associated with a leukaemic blood picture. Since that time much has been written on the association of lymphoblastic or lymphocytic leukaemia and localized malignant tumour formation in lymph nodes, but the complexities of lymph-node histology, the lack of uniform terminology both for cells and for pathological states, and the existence of transitional clinical patterns have prevented any clear-cut and generally acceptable differentiation between leukaemia proper and lymphosarcoma with invasion of the blood.

Gall and Mallory (1942), for example, were often unable to differentiate histologically between the lymph glands from clinically and haematologically leukaemic patients and those from clinically lymphosarcomatous patients, with no blood involvement. They therefore preferred the terms lymphoblastic or lymphocytic lymphoma, and applied them to cases showing respectively immature or mature lymphocytic proliferation in the lymph nodes regardless of whether there were blood changes or not. They did not recognize any special features distinguishing the circulating lymphosarcoma cells from the lymphocytes or lymphoblasts of chronic or acute leukaemia. Many histologists agree with this view of the essential identity of "lymphosarcoma" and leukaemia of the lymphocyte cell series, and regard the relationship of primarily nodal disease to generalized leukaemia as strictly comparable with that of multiple myeloma to plasma cell leukaemia (see also Chapter 16).

On the other hand, many haematologists and most clinicians believe the distinction between lymphosarcoma and leukaemia to be useful and valid, pointing out that the majority of patients with lymphosarcoma never develop leukaemic changes in the blood or bone marrow, that they require a different therapeutic approach and have a different prognosis. Moreover, cytological distinctions have been drawn between the lymphosarcoma cells found in the blood when invasion from a lymphosarcoma does take place and the cells of acute or chronic lymphocytic leukaemia (Wiseman, 1936; Isaacs, 1937; Bethell, 1942). When smears are stained with brilliant cresyl blue and counterstained by a Romanowsky method, the lymphosarcoma cells show a large single nucleolus, eccentrically placed and standing out as a sky-blue area surrounded by a dense rim of nucleolus-associated chromatin, whereas lymphocytes show no nucleolus and lymphoblasts a much less conspicuous one without the heavy chromatin rim. Phase-contrast examination also reveals the much larger and darker nucleolus of the lymphosarcoma cell (Moeschlin, 1949). Apart from the nucleolar differences, lymphosarcoma cells appearing in the blood in patients with the clinical history and signs of lymphosarcoma are usually larger than mature lymphocytes, being commonly 10 to 15 μ in diameter, and possess a more basophilic cytoplasm than that of the lymphocyte, while the nuclear structure, despite the existence of a nucleolus, is coarsely chromatic, and the nuclear outline often kidney-shaped or indented. In cytoplasmic basophilia it resembles a lymphoblast, but the nucleus is too pachychromatic, the nucleolus too large and conspicuous, and the nucleolus-associated chromatin too dense, for the resemblance to be more than superficial.

The frequency with which invasion of the blood stream occurs in lymphosarcoma is impossible to ascertain at present, because of the discrepancies in nomenclature and haematological and histological interpretation referred to above, but it seems likely that perhaps a third of all cases will show this phenomenon, at least terminally (Isaacs, 1937). It is certainly by no means uncommon, since Hauswirth, Rosenow and Lansman (1948) were able to collect more than 150 case reports of the occurrence. In a considerable majority the primary nodal involvement has been mediastinal (Flashman and Leopold, 1929; Bogart, 1946), but many other primary sites have given rise to dissemination, including retroperitoneal and mesenteric lymph nodes, cervical or axillary nodes, and lymphosarcomatous tumours of the tonsils or nasopharynx, in the skin, bones, meninges, intestine, breast, prostate and other organs. There is evidence that irradiation may sometimes stimulate the invasive phase to develop, since the change from non-leukaemic lymphosarcoma to lymphosarcoma cell leukaemia has often been found to follow rapidly after radiotherapy (Kato and Brunschwig, 1933; Hauswirth *et al.*, 1948).

Even during the non-leukaemic phase of lymphosarcoma, when the peripheral leucocyte count is not grossly abnormal, careful examination of the mononuclear cells is likely to reveal a small percentage with the characteristics of lymphosarcoma cells. When the circulation is actively invaded, however, the leucocyte count rises rapidly to levels of 30,000 to 150,000 per cu. mm. and over 90 per cent of the cells may be lymphosarcomatous. Concomitantly with this increase the haemoglobin tends to fall and, later, thrombocytopenia develops. As in chronic lymphocytic leukaemia, there may be an auto-immune haemolytic element in the genesis of anaemia, and when this is so, treatment with corticosteroids may provide relief.

Once the leukaemic phase has developed, the prognosis in lymphosarcoma becomes more grave, the majority of patients dying within a year. Treatment is unsatisfactory, since the response to irradiation, though usually rapid, is very short-lasting, and toxic reactions are often severe. Chemotherapy with antimitotic agents and corticosteroid hormones may be effective in reducing the gland masses and the peripheral leucocyte count but substantial remissions are uncommon. Nevertheless, occasional patients have been known to survive up to 10 years after the onset of the leukaemic phase.

Chronic Monocytic Leukaemia

While granulocytic and lymphocytic leukaemias have clearly recognizable acute and chronic forms, monocytic leukaemia almost invariably runs an acute course and the chronic form of the disease has rarely been observed and reported. Sinn and Dick (1956), in a review of the literature, found a total of only 14 adequately reported cases in which the total duration of the disease was greater than 1 year from the first obvious onset of symptoms or in which the blood and marrow did not show initially evidence of acute leukaemia, monocytic infiltration only becoming prominent after some months' illness. To these cases the authors added 8 of their own. Most patients presented with an initial preleukaemic phase characterized by anaemia, leucopenia, thrombocytopenia and a non-leukaemic bone marrow, either hypoplastic or normally cellular. A progressive but slow deterioration, sometimes punctuated by short remissions, eventually culminated in an acute monocytic leukaemic picture, with a rapid fatal termination.

Cases of this sort are certainly not related to acute monocytic leukaemia as chronic to acute granulocytic, or chronic to acute lymphocytic. They seem to resemble much more closely other acute leukaemias having an unusually prolonged course and perhaps an aplastic or preleukaemic initial phase, and should be regarded as slowly developing acute leukaemias rather than chronic ones.

A more genuine parallel to other forms of chronic leukaemia is shown by the monocyte involvement often seen in chronic granulocytic leukaemia. This monocytic-myelocytic picture, often with 20 to 50 per cent of mature monocytes, seen in an otherwise typical chronic granulocytic leukaemia, provides the "chronic" parallel to acute myelomonocytic leukaemia of the Naegeli type. No distinguishable chronic form of the Schilling type of monocytic leukaemia is known.

Leukaemic Reticulo-endotheliosis

The term leukaemic reticulo-endotheliosis has been applied to cases having the general characteristics of leukaemia, but with hyperplasia of reticulo-endothelial cells in the haemopoietic organs and the appearance of free "reticulum cells" or histiocytes in the blood (Ewald, 1923; Downey, 1938; Fieschi, 1942; Belding, Daland and Parker, 1955; Bouroncle, Wiseman and Doan, 1958).

The disease is extremely rare and difficult to identify, but from a study of 26 cases Bouroncle and her colleagues described it as occurring in adults, chiefly males, and as having an insidious onset with weakness, abdominal pain, fever and a bruising tendency. There was moderate or gross splenomegaly in nearly all cases, and sometimes slight hepatomegaly and lymphadenopathy. Three patients showed skin infiltrates. Clinically the course of the disease varied from acute, with survival less than a year, to very chronic, with survival 16 years.

Haematologically anaemia, leucopenia and thrombocytopenia were usually found, although the leucocyte count was sometimes raised. Bone-marrow aspiration usually proved difficult, but when successful revealed from 30 to 90 per cent reticulum cells. Similar cells were found in variable numbers in the peripheral blood. Histological examination of the viscera *post mortem* showed increases in reticulum cells in many organs, especially the liver and spleen. The lymph nodes and bone marrow also showed extensive infiltration with reticulum cells.

Cases with an acute course clinically resembled acute leukaemia, while those with chronic course simulated myelofibrosis, but the finding of reticulum cells in the blood and bone marrow enabled the diagnosis to be made.

These uncommon forms of leukaemia may represent involvement of cells less differentiated than the myeloblast, lymphoblast or monoblast, namely the primitive free reticulo-endothelial cells, but their precise origin and relation to other myeloproliferative diseases has not yet been determined.

Mixed Leukaemias

Leukaemic proliferation commonly involves more than one cell series; we have seen that both monocytic and myeloblastic cell lines are involved in myelomonocytic leukaemia, and that concurrent erythraemic myelosis may be found in myeloblastic and monocytic

leukaemias, while megakaryocytic hyperplasia often co-exists with chronic granulocytic leukaemia. The mixed occurrences, transitions and overlap seen generally in the myeloproliferative diseases and the comparable interrelationships in the lymphoproliferative diseases will be discussed further in Chapter 16. The term "mixed leukaemia", however, has been used to refer to the combined involvement of myelogenous and lymphogenous tissue in a leukaemic process. It is doubtful whether any of the reported cases of this combination will withstand scrutiny. Those in which adequate haematological details were reported appear to be examples of myeloblastic transformation, mistakenly interpreted as lymphocytic proliferation, in chronic granulocytic leukaemia, or inflammatory polymorphonuclear leucocytosis in chronic lymphocytic leukaemia. If mixed leukaemia in this sense occurs at all, it must be excessively rare.

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CHAPTER 16

THE NATURE AND NOSOLOGY OF LEUKAEMIA

Leukaemoid Reactions and Paraleukaemic States

The nature of leukaemia and its place among related diseases are vexed questions. The acute leukaemias usually differ so sharply from the chronic leukaemias, both in clinical course and haematological manifestations, that it is tempting to regard them as separate diseases; yet intermediate and transitional forms exist. Chronic lymphocytic and granulocytic leukaemias are haematologically distinct, without overlap, yet they are clinically similar in many respects and are further united by their common relationship to the acute leukaemias.

Aplasia of haemopoietic tissue may precede the onset of leukaemia, develop as a temporary phase during the course of the disease, or provide the terminal manifestation, and must certainly, if paradoxically, be regarded as a potentially paraleukaemic disorder.

Myelogenous leukaemias and erythraemias, both acute and chronic, show a close relationship to other myeloproliferative diseases, while lymphogenous leukaemias are similarly related to a perplexing variety of lymphoproliferative states. These relationships are more than superficial, since genuine transitions undoubtedly occur between the leukaemias and supposedly non-leukaemic myeloproliferative and lymphoproliferative states, and intermediate conditions are not rare.

Less closely related to leukaemia are the leukaemoid reactions, in which superficial but sometimes striking resemblances to leukaemia arise during the course of essentially different diseases. Tuberculosis is one such disease, the causative bacillus apparently being capable, in a small proportion of cases, of inducing haematological changes similar to those not only of leukaemia, but also of other myeloproliferative states and of marrow aplasia.

Finally, the relationship of leukaemia to the generality of neoplastic processes requires consideration. This question excited much interest some years ago, before the concept of conditioned and dependent neoplasms became established, but the borders of neoplastic pathology are now so ill-defined that there is ample room for leukaemia to be placed upon them, neither firmly within nor unquestionably without the neoplastic fold.

The relationship of aplasia to leukaemia

The numerous case reports illustrating different aspects of this relationship may be grouped into three categories (Hayhoe, 1957).

1. Transition may occur from an apparently typical aplastic state, with anaemia, leukopenia and thrombocytopenia in the peripheral blood, and an aplastic or hypoplastic marrow showing no increase in primitive cells, to an acute leukaemic picture. The preleukaemic aplasia observed in many closely studied cases appears to have been genuine,

since repeated marrow punctures at different sites during the aplastic phase failed to disclose any increase in primitive cells (Mallarmé, 1949; Williams, 1955; Hayhoe, 1957). Furthermore, a frankly leukaemic picture has not invariably developed within a period of weeks, as might be expected if the supposed aplasia were in reality an aleukaemic leukaemia with hypoplastic or diluted marrow, the primitive cells having been misinterpreted as lymphocytes. In the case reported by Williams the preleukaemic phase extended over a period of 9 years, during part of which a return to a normal haematological picture occurred, while in other cases the aplastic stage was succeeded by a period of several weeks' or months' remission before leukaemic changes became manifest. It is not unlikely that this pattern of events will be observed more commonly now than in the past, since, as Mallarmé (1949) and Block, Jacobson and Bethard (1953) have suggested, many patients who would earlier have died in the preleukaemic phase now survive long enough with antibiotic and steroid therapy to allow the evolution of the disease to be completed.

The separation of preleukaemic aplasia from aplastic cytopenic states with no tendency to convert to leukaemia does not yet appear possible on cytological or clinical criteria. The distinctive cytochemical features of the leucocytes in some forms of leukaemia may prove helpful in this respect, if similar cytochemical characteristics are found in the leucocytes of preleukaemic aplastic states but not in those of non-leukaemic aplasia, but studies in this connection have not yet been reported.

2. Aplastic phases may develop during the course of untreated acute leukaemias, and such phases not uncommonly precede the onset of spontaneous remissions. A very striking example of this sequence was reported by Bassen and Kohn (1952), whose patient had experienced four remissions of acute leukaemia, unassociated with specific anti-leukaemic therapy other than transfusions, and in each case preceded by marrow hypoplasia with peripheral pancytopenia. It is, of course, true that remissions induced by antimetabolites frequently develop after a transitory phase of therapeutically-induced aplasia, and such a preliminary aplastic phase is therefore common to both spontaneous and induced remissions.

While the emergence of peripheral pancytopenia and medullary hypoplasia during the course of acute leukaemia may accordingly be regarded as a hopeful sign that remission may shortly follow, whether the patient is under active treatment or not, this optimistic interpretation is not always justified, for the aplastic state may prove terminal, or relapse to full leukaemia may take place without any interim period of normal haemopoietic activity. When death occurs in an aplastic phase, there may be little histological evidence of leukaemia to be found in the organs and tissues, even though gross leukaemic changes had been present in the blood and bone marrow 2 or 3 weeks before death. Nevertheless, careful study of many sections of the bone marrow will usually reveal some islands of leukaemic tissue. Indeed, Stodtmeister and Buchmann (1941) claimed to have found small nests of leukaemic cells at thorough post-mortem examination in many cases of supposed idiopathic aplastic anaemia of children, and it is probable that these cases were examples of preleukaemic aplasia or of aplasia developing as a terminal feature of undiagnosed acute leukaemia.

3. Cases falling into the third category illustrate a rather different aspect of the relationship between leukaemic changes and marrow failure. They concern peripheral pancytopenias with variable marrow changes which never develop into frank leukaemia although

sometimes showing a substantial increase in primitive cells in the marrow. The cytological stages in the genesis of artificially induced granulocytopenia have been studied by Moeschlin and his associates (1954), who demonstrated that maturation arrest might involve successively more and more primitive stages in the chain of granulocyte development, until arrest at the myeloblast-promyelocyte stage developed, lending the marrow a close resemblance to that of acute myeloblastic leukaemia, although the peripheral blood showed the picture of agranulocytosis. Still later, complete medullary failure ensued. Although neither the spontaneous nor the experimentally induced cytopenias are leukaemic in nature, they are of great interest in showing how a morphological picture like that of leukaemia can result from a non-malignant disturbance of cell maturation, while a further consequence of the same disturbance may be aplastic marrow failure.

Speculation on the nature of leukaemia and its aetiology and pathogenesis must clearly be influenced by the close relationship of leukaemia to aplasia, since hypotheses applicable to the former must relate also to the latter, at least in its paraleukaemic forms.

Leukaemia among the myeloproliferative states

We have seen already that myelogenous forms of leukaemia, that is, those arising from leucocyte precursors in the bone marrow, are frequently associated with concurrent proliferation of other haemopoietic elements in the marrow. The increase in megakaryocytes and the peripheral thrombocytosis commonly observed in chronic granulocytic leukaemia and the not infrequent development of erythroblastosis in acute myeloblastic and monocytic leukaemias illustrate this association. Since Heuck (1879) first drew attention to the association of fibrosis in the bone marrow with a blood picture resembling that of chronic granulocytic leukaemia, the existence of extramedullary haemopoiesis and peripheral leuco-erythroblastosis of varying degrees in myelofibrosis and osteosclerosis came to be increasingly recognized. The extramedullary haemopoiesis or myeloid metaplasia in these conditions was long regarded as a secondary, compensatory phenomenon resulting from overcrowding of the marrow with fibroblastic tissue and the consequent inability of marrow haemopoietic tissue to continue multiplying in the normal site. From careful histological studies of pathological material from cases of myelofibrosis, Vaughan and Harrison (1939) concluded, however, that this explanation was not satisfactory, since extension of haemopoietic sites within the marrow more than compensated for the fibroblastic proliferation and enabled the total erythropoietic and leucopoietic tissue in the marrow to reach or exceed normal amounts. Megakaryocytic proliferation was, indeed, greatly in excess of normal. These authors suggested that some unknown stimulus acting on the primitive mesenchymal reticulum cell might be responsible for proliferation of both its haemopoietic and its fibroblastic derivatives.

This concept of a myelostimulatory factor acting upon the multipotential primitive mesenchymal cells of the marrow or their more differentiated derivatives is paralleled by the concept of myeloproliferative diseases, among which are included not only such well recognized states as myelogenous leukaemias, polycythaemia vera, and myelofibrosis, but also the erythraemic myeloses, megakaryocytic myelosis and all the transitional, intermediate and mixed forms of proliferative disease of the bone-marrow elements (Dameshek, 1951).

The chief evidence in favour of the fundamental interrelationship of these myeloproliferative diseases lies in the innumerable clinical and pathological transitions and overlaps observed between them. Some of the transitional states and mixed proliferations have been mentioned briefly in earlier chapters, but the whole question of the relation of leukaemia to other myeloproliferative disorders is so important that the problem now requires closer consideration.

The cellular elements derived in the bone marrow from the mesenchymal reticulum cell include myeloblasts and granulocytes, erythroblasts and erythrocytes, megakaryocytes and platelets, and fibroblasts and fibrocytes. These elements may proliferate singly, in sequence, or in combination. Under normal conditions fibroblastic activity in the marrow is slight, while the balance of proliferative activity among the other elements is maintained by uncertain physiological mechanisms so that the constitution of the blood remains roughly constant. A sudden change in the peripheral blood calling for haemopoietic activity, such as results from severe haemorrhage, leads to concurrent proliferation of all differentiated marrow elements except fibroblasts, and the development of leucocytosis and thrombocytosis as well as reticulocytosis and increase in red cells. This combined response to physiological stimulation itself suggests that pathological hyperplasia of the marrow cells may be expected to involve more than one cell series in at least a proportion of proliferative disorders.

Polycythaemia vera provides perhaps the best example of mixed and transitional myeloproliferation. Shortly after Vaquez (1892) and Osler (1903) had described the disease as a separate entity involving a great increase in the number of circulating red cells, Türk (1904) observed that the granulocytes were often also substantially increased, and that precursor cells of both red and white cell series were frequently present in the peripheral blood. He suggested that the primary hyperplastic state in the marrow affected leucopoietic as well as erythropoietic tissue. Thrombopoiesis was later observed to be exaggerated, with thrombocytosis in the blood and numerous megakaryocytes in the marrow and in sites of extramedullary haemopoiesis, and the hypothesis that polycythaemia vera is a panmyelosis, involving proliferation of all haemopoietic marrow elements, with erythropoietic activity predominating, became established.

Subsequent studies of many polycythaemic patients throughout the whole course of their disease revealed that the prominent erythrocytosis which originally gave the disease its name was only one aspect of a complex process capable of evolution in diverse though related ways. Descriptive accounts given by such writers as Minot and Buckman (1923), Rosenthal and Bassen (1938), Schwartz and Ehrlich (1950), Dameshek (1950), Wiseman *et al.* (1951), Lawrence, Berlin and Huff (1953) and Wasserman (1954) have greatly clarified the evolutionary sequence and provided a logical explanation of the common patterns of clinical and pathological development seen in this disease.

During the early stages a gradual increase in erythropoietic activity takes place, with expansion of the red marrow throughout the whole skeleton. Accompanying this proliferation a less-marked hyperplasia of granulopoietic and thrombopoietic tissues occurs. When the increase in red cell mass and blood volume has become sufficient to produce symptoms and thus lead to the establishment of the diagnosis, the proliferative process has probably been in force in most cases for several years, and at this time examination of the peripheral blood shows typically a pancytosis, with erythrocytes numbering from

7 to 12 million per cu. mm., leucocytes usually between 10,000 and 40,000 per cu. mm., and platelets commonly more than a million per cu. mm. The predominating leucocytes are neutrophil polymorphonuclear cells, but a few metamyelocytes and myelocytes are nearly always to be seen, and in perhaps 30 per cent of cases the leucocyte count is so high and the immature forms so conspicuous that the blood picture of chronic granulocytic leukaemia is closely simulated. Erythroblasts are also commonly present in small numbers. Bone-marrow aspirates show hyperplasia of all haemopoietic elements, with erythroblasts predominating.

This erythraemic phase of the disease may last many years without substantial change in proliferative emphasis, although as the hyperplastic stimulus continues there is a tendency for extramedullary sites of potential haemopoietic activity to become functional, and the spleen, liver, and sometimes lymph glands enlarge. Splenomegaly may be considerable during the latter part of the erythraemic phase, and the development of marked splenomegaly usually heralds the next evolutionary stage. This involves a gradual failure of erythropoietic activity in the bone marrow, reflected in the peripheral blood by a return to normal of the erythrocyte count and later by a slowly progressive anaemia. The marrow now shows reduction in erythroblasts and conspicuous increase in granulopoietic cells, megakaryocytes, reticulum cells and fibroblasts, a picture intermediate between that of chronic granulocytic leukaemia and that of myelofibrosis. Since the haemoglobin level and red cell count may remain roughly normal for many months during the phase of declining erythropoiesis, this phase appears in a sense as a remission of the polycythaemic process. Nevertheless, leucocytosis and thrombocytosis are usually prominent and precursor cells of both red and white cell series are to be found in the blood, giving a leuco-erythroblastic picture. The cytology of the red cells is generally bizarre, with much anisocytosis and poikilocytosis, and many of them are certainly being formed in extramedullary sites.

Both the erythraemic stage and the stage of partial erythropoietic failure may last several years, and since polycythaemia vera is a disease of the middle-aged or elderly, death may occur from complications of hypervolaemia, or from unrelated causes during one or other of these stages, but some 20 or 30 per cent of patients survive long enough to manifest further evolutionary developments. These are of several possible kinds. Perhaps the commonest arises from the continued overgrowth of fibroblasts in the bone marrow so that marrow aspiration is no longer possible and surgical biopsy reveals gross fibrosis, with numerous megakaryocytes and occasional reticulum cells and myelocytes in the interstices of the fibrous tissue. Hepatosplenomegaly increases greatly, with extramedullary haemopoiesis largely replacing the marrow function, but anaemia becomes inexorably more severe, the high levels of leucocytes and platelets fall, and although leuco-erythroblastosis persists, there is thrombocytopenia and often granulopenia, and death occurs from anaemia, infection or haemorrhage.

Alternatives to the overgrowth of fibroblasts in the marrow with a myelofibrotic termination are predominating proliferations of white cell precursors or megakaryocytes, giving terminal pictures of acute or chronic myelogenous leukaemia, or megakaryocytic myelosis. Patients treated by venesection alone occasionally develop leukaemia in the final stages of their disease, but this complication has appeared to be more common after treatment with radioactive phosphorus. Adequate follow-up studies on large groups of

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at one time granulopoiesis may predominate, at another megakaryocytic hyperplasia, and at another fibrosis.

In the majority of patients with this disease the fibrotic element is most prominent and the condition can be differentiated without difficulty from myelogenous leukaemia, since the leucocyte count in the peripheral blood is not greatly increased, immature cells are not numerous, the bone marrow is intensely fibrotic, and the illness tends to run a prolonged chronic course. Biopsies of the enlarged spleen and liver reveal active extramedullary haemopoiesis without granulopoietic predominance. Among these cases are many where such factors as extrinsic poisons, liver disease, endocrine disease, chronic haemorrhage or haemolysis, or cardiovascular disease may have played some aetiological role, perhaps by causing damage to the bone marrow through direct toxic action or metabolic deficiency, the subsequent hyperplasia of resistant fibroblastic tissue being initially reparative as in hepatic cirrhosis (Wyatt and Somers, 1950).

In other cases, however, the relationship to leukaemia is morphologically and clinically very remarkable, and it may be impossible to differentiate the two diseases, since characteristic features of both may be present at the same time, or may follow one another during the progress of the illness in an individual patient. This relationship is well illustrated by a series of 10 cases reported by Hutt, Pinniger and Wetherley-Mein (1953). Two of the patients proved to have relatively pure disease processes, although the blood findings were initially equivocal. The first presented with splenomegaly and a slight neutrophil leucocytosis accompanied by some granulocytic hyperplasia in the bone marrow, but without anaemia. After 3 years the spleen had enlarged to below the umbilicus, the liver was palpable and the peripheral leucocyte count had risen to 34,000 per cu. mm., chiefly mature polymorphs. Sternal marrow examination and rib biopsy revealed intense hyperplasia of granulocytes with increase in primitive cells and megakaryocytes but no fibrous tissue and no increase in reticulin fibrils, thus confirming the diagnosis of chronic granulocytic leukaemia. The second patient had a rapidly progressive aplastic state, with anaemia, neutropenia and thrombocytopenia, slight generalized lymphadenopathy but no hepatosplenomegaly. A few primitive cells were later found in the peripheral blood and three attempts at marrow aspiration were unsuccessful. Rib biopsy showed replacement of normal structure by proliferating fibroblasts with massive increase in reticulin fibrils. Despite the extreme marrow fibrosis, there was no evidence at autopsy of extramedullary erythropoiesis, and sections of the spleen and lymph nodes showed only some hyperplasia of reticulum cells with increase in reticulin in the interfollicular spaces. The diagnosis of myelofibrosis without myeloid metaplasia was established. The remaining 8 cases showed mixed proliferations of various cell combinations, including reticulum cell hyperplasia and fibrous tissue formation in the marrow of a patient with an otherwise typical chronic granulocytic leukaemia, marked granulocytic and megakaryocytic proliferation in the marrow and spleen of a patient with leuco-erythroblastic anaemia and myelofibrosis, increased granulopoiesis, megakaryocytic hyperplasia and fibrosis of the marrow in the later stages of an initially erythraemic case of polycythaemia vera, and a number of further examples of mixed granulocytic, megakaryocytic and fibroblastic proliferation in patients with no polycythaemic history. The authors emphasized that no convincing evidence of extramedullary erythropoiesis could be found in any case except that of terminal polycythaemia, and they interpreted the foci of granulopoiesis, megakaryocytic hyperplasia,

patients treated before the introduction of P^{32} are not available, but it seems probable that about 10 or 20 per cent of patients treated by venesection and phenylhydrazine developed apparent leukaemia, usually of the chronic granulocytic type. More recent studies on large series of internally irradiated patients show leukaemia incidences of from 10 to 30 per cent, the great majority of the terminal leukaemias being acute myeloblastic (Stroebel, Hall and Pease, 1951; Wiseman *et al.*, 1951; Lawrence *et al.*, 1953; Wasserman, 1954). It is far from certain, however, that irradiation is directly responsible for the increased incidence of acute leukaemia. The tendency for leukaemic changes to develop undoubtedly increases with prolonged survival, and Tinney, Hall and Giffin (1945) found leukaemia in as many as 80 per cent of polycythaemic patients whose disease had lasted over 15 years. Treatment with radioactive phosphorus has been shown to prolong the life-span of patients with polycythaemia vera very substantially and it seems reasonable to suppose that many patients who would earlier have died in the erythraemic phase of the disease now live long enough for the natural myelofibrotic or leukaemic end-stages to develop.

The terminal pathology is usually complex, since even when leukaemic or myelofibrotic changes clearly predominate, proliferation of other cell series is to be found, and fibroblastic hyperplasia accompanies the myeloblastic proliferation in the leukaemic marrow, while myeloblasts may be numerous in the blood and in the marrow in myelofibrosis. Megakaryocytic hyperplasia may be so conspicuous as to dominate the marrow picture and suggest the diagnosis of megakaryocytic myelosis, but again hyperactivity of fibroblastic and granulopoietic tissue is commonly present, while a less striking but quite evident increase in megakaryocytes is invariably present in the myelofibrotic terminal picture and commonly in the leukaemic one.

Although the pattern of development of polycythaemia vera, with its associated and sequential proliferations of the various marrow cell series, usually passes from the erythraemic phase to one of leuco-erythroblastic anaemia or myeloid metaplasia and thence to a fully developed myelofibrosis, myelogenous leukaemia or megakaryocytic myelosis, or to a mixed terminal proliferative state, transitions in a reverse direction have sometimes been observed. Calabresi (1958) collected four examples from the literature and reported a fifth case of chronic granulocytic leukaemia in which a polycythaemic picture developed after radiation treatment of the leukaemia. These cases, though rare, provide further support for the fundamental similarity of leukaemic and erythraemic myeloproliferation.

The terminal stages of polycythaemia vera in which marrow fibrosis and megakaryocytic hyperplasia predominate are closely similar both clinically and haematologically to many cases reported variously under the names myelosclerosis, myelofibrosis, aleukaemic megakaryocytic myelosis, agnogenic myeloid metaplasia, myeloid megakaryocytic hepatosplenomegaly and chronic non-leukaemic myelosis (see also p. 297). Despite the confusing variety of titles, these cases appear to be fundamentally similar. They show proliferation of fibroblasts and megakaryocytes in the marrow, enlargement, often very great, of spleen and liver, deficient red cell formation and a leuco-erythroblastic anaemia, with varying numbers of immature red and white cell precursors in the peripheral blood. As we have seen, this is precisely the picture to be observed in the later stages of many cases of polycythaemia, and the variations in proliferative emphasis between different marrow elements already described in polycythaemia are also found in the non-polycythaemic cases, so that

at one time granulopoiesis may predominate, at another megakaryocytic hyperplasia, and at another fibrosis.

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reticulum cell proliferation and fibrosis found in the spleen, liver, lymph nodes and sometimes in the kidneys and suprarenals in most of their cases as manifestations of the fundamental proliferative process and not as sites of compensatory haemopoiesis secondary to reduced marrow activity. These views are in accord with those earlier expressed by Heller, Lewisohn and Palin (1947), who concluded that idiopathic myeloid metaplasia, whether associated with marrow fibrosis or not, was an unusual variant of granulocytic leukaemia.

It is but a short step from the leuco-erythroblastosis, sometimes with considerable numbers of myeloblasts, seen in myelofibrosis or in the terminal stages of polycythaemia vera, to acute erythroleukaemia with its mixed or consecutive erythroblastic and leukaemic proliferations. Indeed, Di Guglielmo, in many of his publications on erythraemic myelosis (see p. 289), described the varying combinations of leukaemia and polycythaemia as "complete" erythroleukaemia, and the combinations of acute leukaemia and anaemic erythraemic myelosis as "incomplete" erythroleukaemia. Martin and Bayrd (1954) also regarded these two manifestations of combined erythropoietic and leucopoietic hyperplasia as respectively "chronic" and "acute" bidirectional responses of the common precursor cell to a proliferative stimulus. It should be added, of course, that in the "chronic" form third and fourth lines of proliferation may be active, since megakaryocytic and fibroblastic hyperplasia may also be found.

The existence of all these clinical and haematological syndromes, with mixed and transitional proliferations affecting reticulum cells, erythroblasts and erythrocytes, myeloblasts and granulocytes, megakaryocytes and platelets, and fibroblasts and fibrocytes, in various combinations and alternations, strongly supports the concept that the primitive multipotential reticulum cell is in every case responding to some unknown stimulus in a fundamentally similar manner, though with varying progenitive emphasis. That the hypothetical myelostimulatory principle might conceivably be hormonal in character has been suggested by Dameshek (1951), who pointed to the recognized marrow stimulation following the administration of adrenocorticotrophic hormone, and the myeloid metaplasia resulting from continued injection of crude pituitary extracts and testosterone (Selye and Stone, 1950).

Although the evidence so far reviewed appears convincing, certain biochemical observations have been interpreted as indicating that the similarities between the myeloproliferative diseases are superficial rather than fundamental. These observations concern the leucocyte alkaline phosphatase activity in different members of the myeloproliferative group. Studies by both chemical and cytochemical methods have demonstrated that the leucocytes of chronic granulocytic leukaemia contain very little alkaline phosphatase, whereas in polycythaemia vera and other non-leukaemic myeloproliferative states the granulocytes are very rich in the enzyme, even when the peripheral leucocyte count is leukaemoid in character (Wachstein, 1946; Valentine and Beck, 1951; Valentine *et al.*, 1952; Wiltshaw and Moloney, 1955). The contrast is a very striking one, and it would be hard to avoid the conclusion that leukaemic granulocytes are essentially different from the proliferating granulocytes of polycythaemia, myelofibrosis and other states of myeloid metaplasia, were it not for the existence once more of intermediate and transitional biochemical findings. Thus Valentine and his associates (1952) found occasional instances of low alkaline phosphatase content in the leucocytes of myeloid metaplasia, and Mitus

and his colleagues (1958*a*) reported similar findings in 7 of 23 cases, the low levels being found in cases with prominent cellular immaturity in the peripheral blood. The latter authors argued that the cytochemical character of the leucocytes in these cases provided a link between the relatively benign leucocytic proliferation of polycythaemia and myeloid metaplasia and the more clearly malignant process of chronic granulocytic leukaemia. Cytochemical transformation does appear sometimes to run parallel with the clinical and haematological evolution observed among these diseases. Osgood, Seaman and Koler (1957) described 2 patients with polycythaemia, having high leucocyte alkaline phosphatase levels, who subsequently developed chronic granulocytic leukaemia, with phosphatase negative cells. In a third case the transformation was histologically complete but the leucocytes remained strongly positive throughout. Under treatment with X-rays or alkylating agents patients with chronic granulocytic leukaemia have been reported to show a change in leucocyte alkaline phosphatase activity from initially very low levels to levels at the lower range of normal, and in very full remission high normal levels of enzyme have occasionally been found (Hayhoe and Quaglino, 1958; Mitus *et al.*, 1958*a*). The enzymatic differences do not therefore present an insuperable barrier to the acceptance of the myeloproliferative concept, but they do suggest that complete transitions between the related syndromes and genuine mixtures of leukaemic proliferation with hyperplasia of other cell lines derived from the primitive reticulum cell are much less common than might be supposed from simple histological appearances.

Leukaemia and the lymphoproliferative states

Just as the proliferative diseases involving myeloid derivatives of the primitive mesenchymal cell are closely related to one another, so the proliferative states of lymphoid derivatives of the same multipotential cell are similarly interrelated. Here, once again, considerable confusion of nomenclature, arising from differences in interpretation and emphasis between clinicians, pathologists working with fixed tissue sections, and haematologists studying smears and imprint preparations, for long tended to obscure the essential unity of the lymphoproliferative diseases. While differences in classification still abound, there now appears to be general agreement that the primary tumours of lymphoid tissues, sometimes known as the "malignant lymphomas" or "lymphoblastomas", are all fundamentally related to one another and to the lymphogenous leukaemias (Warthin, 1931; Ginsburg, 1934; Warren and Picena, 1941; Gall and Mallory, 1942; Herbut, Miller and Erf, 1945; Jackson and Parker, 1947; Custer and Bernhard, 1948; Berman, 1953; Lumb, 1954; Berman, 1957). The lymphoid progeny of the primitive mesenchymal cell include reticulum cells, various lymphocyte precursors, mature lymphocytes and occasionally fibroblasts, and the tumours arising from lymphoid tissue may be composed predominantly of a single cell line either well or poorly differentiated, or of mixed cell lines. The more common and clearly defined clinical and histological patterns are generally classified, though with varying nomenclatures, in accordance with the cytological nature and degree of maturity of the predominating cells. Thus tumours composed chiefly of reticulum cells are called reticulum cell sarcomas or lymphomas, those made up of lymphocyte precursors are lymphoblastic lymphomas, lymphoblastic reticulosarcomas, lymphoblastic lymphosarcomas or undifferentiated lymphosarcomas, those having predominantly mature

reticulum cell proliferation and fibrosis found in the spleen, liver, lymph nodes and sometimes in the kidneys and suprarenals in most of their cases as manifestations of the fundamental proliferative process and not as sites of compensatory haemopoiesis secondary to reduced marrow activity. These views are in accord with those earlier expressed by Heller, Lewisohn and Palin (1947), who concluded that idiopathic myeloid metaplasia, whether associated with marrow fibrosis or not, was an unusual variant of granulocytic leukaemia.

It is but a short step from the leuco-erythroblastosis, sometimes with considerable numbers of myeloblasts, seen in myelofibrosis or in the terminal stages of polycythaemia vera, to acute erythroleukaemia with its mixed or consecutive erythroblastic and leukaemic proliferations. Indeed, Di Guglielmo, in many of his publications on erythraemic myelosis (see p. 259), described the varying combinations of leukaemia and polycythaemia as "complete" erythroleukaemia, and the combinations of acute leukaemia and anaemic erythraemic myelosis as "incomplete" erythroleukaemia. Martin and Bayrd (1954) also regarded these two manifestations of combined erythropoietic and leucopoietic hyperplasia as respectively "chronic" and "acute" bidirectional responses of the common precursor cell to a proliferative stimulus. It should be added, of course, that in the "chronic" form third and fourth lines of proliferation may be active, since megakaryocytic and fibroblastic hyperplasia may also be found.

The existence of all these clinical and haematological syndromes, with mixed and transitional proliferations affecting reticulum cells, erythroblasts and erythrocytes, myeloblasts and granulocytes, megakaryocytes and platelets, and fibroblasts and fibrocytes, in various combinations and alternations, strongly supports the concept that the primitive multipotential reticulum cell is in every case responding to some unknown stimulus in a fundamentally similar manner, though with varying progenitive emphasis. That the hypothetical myelostimulatory principle might conceivably be hormonal in character has been suggested by Dameshek (1951), who pointed to the recognized marrow stimulation following the administration of adrenocorticotrophic hormone, and the myeloid metaplasia resulting from continued injection of crude pituitary extracts and testosterone (Selye and Stone, 1950).

Although the evidence so far reviewed appears convincing, certain biochemical observations have been interpreted as indicating that the similarities between the myeloproliferative diseases are superficial rather than fundamental. These observations concern the leucocyte alkaline phosphatase activity in different members of the myeloproliferative group. Studies by both chemical and cytochemical methods have demonstrated that the leucocytes of chronic granulocytic leukaemia contain very little alkaline phosphatase, whereas in polycythaemia vera and other non-leukaemic myeloproliferative states the granulocytes are very rich in the enzyme, even when the peripheral leucocyte count is leukaemoid in character (Wachstein, 1946; Valentine and Beck, 1951; Valentine *et al.*, 1952; Wiltshaw and Moloney, 1955). The contrast is a very striking one, and it would be hard to avoid the conclusion that leukaemic granulocytes are essentially different from the proliferating granulocytes of polycythaemia, myelofibrosis and other states of myeloid metaplasia, were it not for the existence once more of intermediate and transitional biochemical findings. Thus Valentine and his associates (1952) found occasional instances of low alkaline phosphatase content in the leucocytes of myeloid metaplasia, and Mitus

of acute lymphoblastic leukaemias first manifest themselves with glandular enlargement before changes have occurred in the blood and bone marrow, and although the histological appearance of biopsied material may resemble that of lymphoblastic lymphoma, the cytology of aspiration or imprint smears is unmistakable and the full picture of acute leukaemia inevitably develops.

The reticulum cell sarcomas are rarely associated with leukaemic changes, but occasionally cells of histiocytic or monocytoid appearance are found in the peripheral blood and bone marrow, giving the picture of leukaemic reticulo-endotheliosis (p. 301) or the Schilling type of monocytic leukaemia. Similar leukaemic changes may rarely occur in Hodgkin's sarcoma, but other mixed forms of lymphoma, including Hodgkin's disease and paragranuloma, do not exhibit leukaemic phases.

Chronic lymphocytic leukaemia, its distinctive variant lymphosarcoma cell leukaemia, and the uncommon diseases, leukaemic reticulo-endotheliosis and the pure monocytic form of leukaemia, appear therefore to be closely linked with the non-leukaemic malignant lymphomas so far as clinical and simple pathological evidence is concerned. Such histochemical studies as have been carried out lend support to the concept of unity among the lymphoproliferative diseases, since no striking differences between the cytochemistry of lymphocytes from leukaemia and those from lymphomas have been found with the periodic acid-Schiff reaction for glycogen, and the ranges of granulocyte alkaline phosphatase have been similar in both lymphocytic leukaemia and malignant lymphomas (Hayhoe and Quaglino, 1958; Mitus *et al.*, 1958*b*; Quaglino and Hayhoe, 1959).

At this point consideration may also be given to macroglobulinaemia and multiple myeloma, since both these disorders have some relationship to lymphocytic leukaemia, although neither falls very clearly into the lymphoproliferative group of disorders.

Macroglobulinaemia was first described by Waldenström in 1944 and the syndrome soon came to be widely recognized. More than 50 case reports have now been published and the clinical and pathological features of the disorder have been several times reviewed (Mackay *et al.*, 1956; Bousser and Boivin, 1957). The essential abnormality in this disease is a derangement of serum proteins, the pattern being dominated by a component of molecular weight in the region of 1,000,000 with ultracentrifugal sedimentation constant about 17–20 S_{20} (Svedberg units). This high molecular weight protein was named macroglobulin or the 20' component by Waldenström; small amounts may be present in normal sera and moderate increases have been found in various dysproteinaemic diseases, but these are regarded as symptomatic increases and do not compare in quantity with the excessive amounts present in macroglobulinaemia proper (Waldenström, 1958). The simplest test for macroglobulins is the Sia water dilution test; a heavy white precipitate is usually produced when a few drops of serum containing the abnormal globulin are placed in a cylinder of water. If this test is positive, confirmation of the nature of the precipitable component may be sought by more elaborate methods. Unfortunately the water dilution test is not reliable, since it may be positive in the absence of macroglobulins and negative in their presence, but it does serve as a rough preliminary screening test if its lack of specificity is appreciated. The electrophoretic mobility of macroglobulins ranges between that of beta and gamma globulins, but the pattern is not distinguishable from that of myelomatosis, and ultracentrifugal studies are really necessary to establish the diagnosis. The clinical picture in macroglobulinaemia is one of chronic, slowly pro-

lymphocytes are lymphocytic lymphomas or lymphosarcomas, and if the differentiation is very well marked with follicular proliferation, follicular lymphomas. When both lymphocytic and reticulum cell elements are strongly represented, giving a mixed picture, the appearance is that of Hodgkin's disease, while mixed proliferations with many lymphocytes and relatively small numbers of reticulum cells have been classified as Hodgkin's paragranuloma or reticular lymphoma, and mixtures with a predominance of reticulum cells and few lymphocytes have been called Hodgkin's sarcoma, although they are virtually indistinguishable from reticulum cell sarcoma. Rarely, fibroblastic proliferation may be conspicuous enough to give the appearance of fibrosarcomas. Between these arbitrarily defined conditions transitions frequently occur. Custer and Bernhard (1948) found a virtually complete alteration in the histological pattern of the tumour in over 30 per cent of 207 cases of Hodgkin's disease in which sequential biopsies or biopsy and autopsy findings could be compared, and similar alterations and transitions were common among other varieties of lymphoma. Mixed forms of disease are even more frequently observed; among 700 cases studied by Custer and Bernhard, no fewer than 384 showed combined lesions, the histological appearances of different varieties of lymphoma being present in different foci in the same individual, or even in neighbouring areas in the same lymph node. The transitions and variations are particularly well exemplified by follicular lymphoma, which initially shows a well-marked pattern of lymphocytic and follicular differentiation, but after an unpredictable period undergoes a transformation to differentiated lymphocytic lymphoma, perhaps with leukaemia, or to lymphoblastic lymphoma, or to reticulum cell sarcoma, or to Hodgkin's disease, or to various combinations of these conditions (Symmers, 1938, 1942, 1948; Wetherley-Mein *et al.*, 1952). This situation, which closely resembles that existing among the members of the myeloproliferative disease group, is to be expected, since all the cell lines involved are once more common derivatives of the multipotential mesenchymal cell.

The peripheral-blood picture of chronic lymphocytic leukaemia, with relative and absolute lymphocytosis, is found at some stage of the disease in a high proportion of patients with well-differentiated lymphocytic lymphoma or lymphosarcoma. A leukaemic blood picture occurred in 48 per cent of such patients in the series of Gall and Mallory (1942), and Berman (1953) suggested that lymphocytic lymphoma and chronic lymphocytic leukaemia were virtually identical diseases, varying only in the relative degrees of lymph-node hyperplasia and blood and bone-marrow involvement. A blood picture somewhat resembling that of acute or chronic lymphocytic leukaemia may develop during the course of the less differentiated "lymphoblastic" lymphoma or lymphosarcoma. The incidence was given by Gall and Mallory as 38 per cent and by Berman as 50 per cent. Probably most of the "lymphosarcoma cell" leukaemias or "leukosarcomas", with cells in the peripheral blood having the peculiar cytological characters earlier described (p. 299), which render them distinct both from mature lymphocytes and from the customary lymphoblasts of acute lymphoblastic leukaemia, are examples of blood involvement in this variety of lymphoma. The lymph-node infiltration in acute lymphoblastic leukaemia is not paralleled by any non-leukaemic malignant lymphoma; the leukaemic lymphoblast is clearly different in smear and imprint preparations from the lymphoid reticulum cell, prolymphocyte or "lymphoblast" of normal lymph nodes and its proliferating counterpart in lymphoblastic lymphoma (Sundberg, 1947; Berman, 1957). Though a small proportion

of acute lymphoblastic leukaemias first manifest themselves with glandular enlargement before changes have occurred in the blood and bone marrow, and although the histological appearance of biopsied material may resemble that of lymphoblastic lymphoma, the cytology of aspiration or imprint smears is unmistakable and the full picture of acute leukaemia inevitably develops.

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gressive anaemia with lassitude and dyspnoea on exertion, moderate generalized lymph-node enlargement, sometimes hepatosplenomegaly, and, very characteristically, episodes

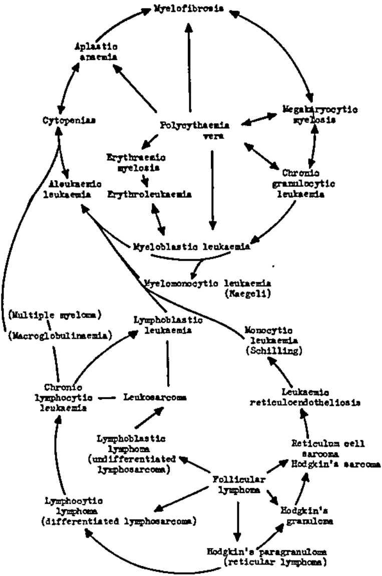

FIG. 53. The leukaemias among the myelo proliferative, lymphoproliferative and aplastic states.

of bleeding from the mouth and nose. In many patients marked engorgement of retinal veins and scattered retinal haemorrhages have been observed.

The peripheral-blood changes are not striking, but the total leucocyte count may be raised a little above the normal range and the percentage of lymphocytes in the differential count is commonly high. Rarely, macroglobulinaemia has been found with the peripheral-blood picture of chronic lymphocytic leukaemia. The cytology of the bone marrow is characteristic. Lymphocytic mononuclear cells, with small amounts of easily fragmented cytoplasm and a marked tendency to smear into Gumprecht shadows, predominate. This persistent and conspicuous marrow lymphocytosis, though not associated often with leukaemic changes in the blood, establishes a relationship with chronic lymphocytic leukaemia, which is further strengthened by the existence of generalized lymphadenopathy. In some cases the sternal marrow has contained a large minority of plasma cells, and tissue mast cells have often been conspicuous (Tischendorf and Hartmann, 1950). Examples of macroglobulinaemia have, however, been observed in patients with a normal bone marrow, but with changes in the lymph glands resembling lymphosarcoma.

The abnormal proteins found in multiple myeloma are not macroglobulins, and although plasmacytosis may be considerable in the marrow of patients with macroglobulinaemia, destructive bone lesions are rare. The general clinical and pathological patterns of myelomatosis and macroglobulinaemia show many similarities, however, and transitional forms between the two diseases probably occur.

The same is true of multiple myeloma and lymphocytic leukaemia; abnormal proteins suggestive of myeloma, and Bence-Jones proteinuria, may occur in chronic lymphocytic leukaemia, though they are very rare findings. Certainly the similarities and transitions observed between lymphosarcoma, lymphocytic leukaemia, macroglobulinaemia and multiple myeloma suggest that both the latter diseases should be tentatively classified among or in close relation to the lymphoproliferative states. Moreover, selective cytopenias or pancytopenia are not uncommon in macroglobulinaemia, so that an association with the aplastic states exists. It seems probable in retrospect that many atypical examples of myelomatosis, lymphocytic leukaemia, lymphosarcoma or pancytopenia may actually have been cases of macroglobulinaemia (Mackay *et al.*, 1956).

In Fig. 53 the relationships between the leukaemias, the aplastic states, the myeloproliferative syndromes and the lymphoproliferative diseases are represented diagrammatically, the arrows indicating the directions in which transitions have been observed to occur.

Leukaemoid Reactions

Since its introduction by Krumbhaar (1926), the term "leukaemoid" has been applied to changes in the blood and haemopoietic organs resembling those of one or other form of leukaemia, but arising during the course of non-leukaemic diseases. It will be clear from the preceding pages that the haematological findings in the myeloproliferative and lymphoproliferative syndromes provide a wide borderline between leukaemic and leukaemoid pictures, where it may be difficult to decide which term is the more appropriate, but we shall now be concerned with the unquestionably leukaemoid reactions occasionally observed in association with certain infections, intoxications, metastasizing tumours, and some non-leukaemic blood diseases. Very many examples of such reactions have been reported, some distinguishable from leukaemia only when post-mortem examination revealed absence of leukaemic infiltrations and the presence of another pathological

process, others resembling leukaemia merely in the height of the peripheral leucocyte count or in the presence of a few leucocyte precursors in the circulation. The extensive earlier literature on leukaemoid reactions was fully reviewed by Forkner (1938), and later reviews include those of Heck and Hall (1939), Hill and Duncan (1941) and Hilts and Shaw (1953).

In most cases the differentiation from leukaemia is not difficult when the clinical findings and the results of blood and bone-marrow examination are considered together. The high alkaline phosphatase level in the granulocytes of most leukaemoid reactions contrasts sharply with the very low level of this enzyme in the neutrophils of granulocytic leukaemias and this difference may be very helpful. Another criterion of general value in separating acute leukaemias from comparable leukaemoid reactions is the magnitude of the platelet count, since severe thrombocytopenia is much more likely to be associated with leukaemia than with a leukaemoid reaction.

Infections. Polymorphonuclear leucocytosis with an increase in the proportion of stab cells and occasional myelocytes in the circulation is, of course, a common feature of many bacterial infections, and from time to time exceptionally high total counts with considerable numbers of granulocyte precursors, including even some promyelocytes and myeloblasts, have been observed. Most of the reported examples of leukaemoid reactions resembling chronic granulocytic leukaemia in the morphological appearances of the peripheral blood are explicable as exaggerated responses to severe infection. They include cases of pneumonia, meningitis, septicaemia, diphtheria, and plague, and in each of these conditions leucocyte counts between 70,000 and 120,000 per cu. mm. have been observed, with varying numbers of granulocyte precursors. The diagnosis has usually been clinically obvious, however, and there has been little real confusion with leukaemia.

A blood picture very like that of chronic lymphocytic leukaemia may be seen in whooping-cough, chicken-pox, measles and infectious lymphocytosis, with mature lymphocytes numbering more than 50,000 and even sometimes more than 100,000 per cu. mm. in the peripheral blood. Once again, the clinical picture is usually diagnostic and confusion with leukaemia is unlikely. Infectious mononucleosis may be said to show a leukaemoid reaction, but the abnormal lymphocytes are of a characteristic type, anaemia and thrombocytopenia are very rare, and there is seldom any prolonged difficulty in diagnosis.

Leukaemoid reactions more closely resembling various forms of leukaemia and presenting serious diagnostic problems have most often been encountered in association with tuberculosis, and the special relation of this disease to the leukaemic, myeloproliferative, lymphoproliferative and aplastic states is discussed separately below.

Intoxications. Granulocytic leukaemoid patterns have been reported to occur in mustard-gas poisoning (Krumbhaar, 1926), in mercury poisoning (Downey, Major and Noble, 1930) and during the administration of sulphonamides (Whittemore and Stich, 1942; Kracke, 1944), and marked leucocytosis may develop in eclampsia. Such reactions are extremely rare.

Disseminated malignant metastases. When metastatic deposits occur in the bone marrow as a consequence of the spread of a primary malignant tumour from the breast, prostate, thyroid, kidney or elsewhere, a leuco-erythroblastic picture with anaemia, immature cells of both red and white cell series, and sometimes thrombocytopenia, commonly develops in the peripheral blood. There is often a leucocytosis, sometimes high

enough to suggest leukaemia when taken in association with the presence of granulocyte precursors in the circulation. Since the clinical picture is one of severe illness and neither the primary tumour nor secondary deposits may be obvious, confusion with acute or chronic granulocytic leukaemia may well occur; the leucocyte alkaline phosphatase content provides a valuable diagnostic pointer, since it is high in disseminated malignancy and low in granulocytic leukaemias. Leukaemoid pictures in malignancy have sometimes developed in the absence of obvious bone-marrow metastases, and lymphocytic rather than myelocytic cells have occasionally been prominent (Meyer and Rotter, 1942; Bichel, 1949), but these events are extremely rare, leukaemoid reactions in malignancy being almost invariably myeloid exaggerations of the leuco-erythroblastic response to bone-marrow invasion in the terminal stages of the disease. It is possible, as Bichel acknowledged, that the few cases described as lymphocytic leukaemoid reactions in disseminated carcinoma may have been examples of coincidence of malignancy and leukaemia, in which either the malignant process itself or the radiotherapy directed against it had exerted a strong antagonistic action upon the leukaemic proliferation, so that few signs of leukaemic infiltration remained at the time of post-mortem examination.

Non-leukaemic blood disorders. Marked leucocytosis with a shift to the left in the granulocyte series frequently follows severe haemorrhage or haemolysis, and the total leucocyte count may reach 60,000 to 100,000 per cu. mm. The blood picture shows predominantly mature segmented and stab cells, however, and myelocytes rarely make up more than 5 to 10 per cent of the total. Such an increase in granulopoietic activity, associated with erythropoietic hyperplasia, may reach leukaemoid proportions in severe dyshaemopoietic anaemias, especially megaloblastic ones, during phases of spontaneous or therapeutically induced remission. A few myelocytes are commonly seen in the blood in Addisonian pernicious anaemia and in megaloblastic anaemia of pregnancy (Callender, 1944) and leucocyte counts of 20,000 to 40,000 per cu. mm., including as many as 50 per cent of myelocytes and even a small percentage of myeloblasts, have been reported occasionally as leukaemoid reactions (Heck, 1932; Ritchie, 1952; Sclare and Cragg, 1958). Perhaps the haemopoietic substances, especially folic acid and crude liver extracts, given in the treatment of some of these primary megaloblastic states contributed to the development of a leukaemoid picture by their known accelerating action on granulopoiesis.

Leukaemoid reactions may of course result from the combined effect of more than one of these stimulating causes. Thus the leuco-erythroblastic response to terminal malignancy with marrow invasion may be associated with exaggeration of the granulocytic component in response to respiratory or urinary infection, while haemorrhage from the alimentary tract or elsewhere may lead to increase in both erythropoietic and granulopoietic activity in the bone marrow, reflected in both components of the leuco-erythroblastic picture.

Tuberculosis. The important place of disseminated tuberculosis among processes capable of inducing leukaemoid reactions has long been recognized. A genuine combination of tuberculosis and leukaemia is not uncommon, though probably no greater than might be expected from chance association, but since the early reports of Roth (1913) and Marshall (1915), a considerable number of cases have been described in which the blood and bone-marrow picture of acute or chronic leukaemia was observed during life, yet no leukaemic infiltration was found at autopsy, extensive miliary tuberculosis being present instead. These leukaemoid reactions have not been uniform in type, but have suggested

granulocytic (Custer and Crocker, 1932; Leibowitz, 1938; Friend and Thackray, 1952), lymphocytic (Landon, 1925; Gardner and Mettier, 1949) or monocytic (Gibson, 1946) forms of leukaemia, and have most often resembled acute or subacute varieties of the disease. Aleukaemic blood findings in a lymphocytic leukaemoid reaction to tuberculosis were described by Staffurth and Spencer (1950), and features like those of acute aleukaemic myeloblastic leukaemia were observed in the blood and bone marrow as a transient phenomenon during the course of atypical disseminated tuberculosis in a patient observed by Medd and Hayhoe (1955).

In view of the close association of leukaemias with aplastic, myeloproliferative and lymphoproliferative diseases, it is particularly interesting to note that the haematological complications of tuberculosis include not only leukaemoid reactions, but also myelofibrosis with leuco-erythroblastic anaemia and megakaryocytic hyperplasia, polycythaemia vera, and peripheral pancytopenias with either normally cellular or hypoplastic marrow, but without fibrosis. The remarkable range of these abnormalities and their close resemblance to the primary myeloproliferative and aplastic states have been discussed by Medd and Hayhoe (1955) and Hayhoe (1957). Tuberculous myelofibrosis is clinically and haematologically very like the idiopathic form of this disease. Crail, Alt and Nadler (1948) described four such cases and collected from published reports seven others, in all of which acute caseating tuberculosis was considered to be responsible for the bone-marrow fibrosis. In a footnote to their paper, however, they mentioned a patient with a leuco-erythroblastic anaemia, who was found at autopsy to have miliary tuberculosis and a hypoplastic marrow without fibrosis. Leucopenia and pancytopenia in acute tuberculous septicaemia were observed by Ball, Joules and Pagel (1951) in three patients, and they collected eight similar cases from the literature. Four further cases of pancytopenia in miliary tuberculosis were reported by Medd and Hayhoe (1955), and in one of them consecutive phases of pancytopenic hypoplasia, aleukaemic myeloblastic leukaemoid reaction, and finally medullary aplasia were observed, illustrating the close relationship of cytopenic and leukaemoid reactions to tuberculosis. Blood pictures characteristic of polycythaemia vera but believed to be secondary to miliary tuberculosis were described by Fitzpatrick and Schwartz (1949) and by Guild and Robson (1950), while Tsévrénis, Thuilliez and Panas (1951) reported a reaction resembling erythraemic myelosis in tuberculosis.

The fact that such a wide range of leukaemoid, cytopenic and myeloproliferative responses may occur during the course of acute tuberculosis further supports the conception that the corresponding primary blood disorders are themselves closely related. It seems reasonable to assume that the tuberculous cases have a common pathogenesis, the factors active upon the haemopoietic system being products of the tubercle bacillus and the mechanism of action perhaps involving hypersensitivity. In nearly all reported cases of tuberculous blood dyscrasias the disseminated tuberculosis has been atypical histologically, being characterized by multiple necrotic foci in various organs with little surrounding cellular reaction and few giant cells, epitheloid cells, or lymphocytes. Large numbers of organisms have sometimes been found in these lesions which markedly resemble the kind of acute caseating miliary tuberculosis produced experimentally by Rich (1944) by the injection of large doses of bacilli into the blood stream of hypersensitive animals. A further experimental parallel to the leukaemoid type of reaction is provided by the

myelocytic-leukaemoid response in the blood of tuberculous rabbits given injections of tuberculin, a response presumably mediated by an allergic mechanism (Feldman and Stasney, 1937). Moreover, Sabin (1932) found that a striking multiplication of fibroblasts could be caused, both locally and diffusely, by an unsaponified higher-alcohol derivative of the waxes of tubercle bacilli, an observation which may be relevant to the pathogenesis of tuberculous myelofibrosis.

Since this whole range of haematological complications of tuberculosis appears therefore to have a single cause and common pathogenetic mechanism, it becomes the more likely that a comparable state of affairs should exist for the parallel idiopathic group of blood diseases.

Interesting and informative though these haematological reactions to tuberculosis may be, it should be emphasized that they are relatively uncommon; leukaemia and other related diseases may co-exist with tuberculosis as primary disease states, and the blood picture should be interpreted as secondary to tuberculosis only when atypical features, response to anti-tuberculous therapy, or absence of characteristic infiltrations at autopsy make the double diagnosis unlikely.

The nature of leukaemia and its relation to neoplastic processes

The proliferation of leucocytes and their precursor cells in leukaemia has commonly been regarded as a neoplastic process of the leucopoietic system, comparable to the growth of malignant tumours from other tissues in the body. The arguments in support of this view are many. Leukaemic hyperplasia is invariably fatal; the proliferating cells show cytological abnormalities; numerous mitotic figures occur, some of them irregular; extensive infiltrations of non-haemopoietic organs and tissues develop, similar to metastases of malignant tumours; although the leukaemias are almost always widely generalized diseases from the earliest stages as far as can be ascertained, an extremely close relation exists between certain forms of leukaemia and such localized processes of tumour formation as chloroma and solitary myeloma, while lymphosarcoma and reticulum cell sarcoma afford a bridge between leukaemia and the generality of malignancies. Transmission experiments in mouse leukaemias have long established that the affected cells are capable of reproducing the disease when introduced into healthy animals, in a manner similar to that of other malignant tumour cells.

Against these powerful arguments opposing ones have been advanced. Although leukaemia is inevitably fatal, remarkably complete remissions sometimes occur spontaneously and can occasionally be induced by such "physiological" mechanisms as blood transfusion or the development of infection; such remissions have few counterparts in malignant states. The abnormal cytological and mitotic features of leukaemic cells resemble those of malignant tumours, but they can all be duplicated in leukaemoid states and in conditions known to be non-malignant, such as pernicious anaemia. The infiltrations of leukaemia chiefly represent vascular permeation of organs with circulating cells, which show little tendency to invade and destroy local tissues as metastases do; since the cells concerned are often immature, they may be expected to exhibit further proliferative activity at sites of deposition. The transplantability of animal leukaemias contrasts sharply with the failure to transmit human leukaemias.

Additional arguments for and against the neoplastic nature of leukaemia concerned the

results of *in vitro* culture experiments, some interpreting the very conflicting findings as evidence that leukaemic cells were essentially normal and capable of maturing normally *in vitro*, whereas others believed the evidence more in favour of a permanent malignant change in the leukaemic cell. Discussion over the neoplastic nature of leukaemia was still being forcefully pursued in quite recent years (Whitby, 1951; Furth, 1951), but an increasing awareness of the multiple factors involved in cell proliferations has undermined the position of both protagonistic schools. Furth (1953, 1954) reassessed the character of leukaemias in the light of experimental studies on dependent or conditioned as contrasted with autonomous neoplasms. He pointed out that unrestrained proliferation was not necessarily a distinguishing character of neoplastic cells, since most normal cells were capable of similar proliferation in tissue culture. Clearly the balance of cell multiplication in the normal organism might reasonably be expected to be affected by restraining and stimulating physiological mechanisms. Imbalance might result from many possible circumstances external to the cell, as well as from primary genetic alterations in the cell itself or alterations due to the action of intracellular agents. The forces operating on cell proliferation have been very inadequately elucidated as yet, although the role of certain hormones in controlling cell growth in their target organs and in relation to malignant change in these organs has been partially explored.

Malignant tumours have been produced experimentally by altering the hormone–target organ relationship, as by destroying the thyroid gland and thus stimulating excessive production of thyroid-stimulating hormone with the eventual formation of pituitary tumours as a result of sustained proliferation of cells concerned with TSH secretion. Such tumours can be transplanted into thyroid-deficient animals, where they grow and metastasize in a malignant fashion, yet their growth can be controlled, both in the original animal and in the host, by administration of thyroid hormone. After prolonged growth these dependent tumours often become autonomous and no longer respond to thyroid control (Furth and Burnett, 1951; Gadsden and Furth, 1953).

The effects of hormone treatment of prostatic carcinoma in man and of some forms of mammary carcinoma illustrate the importance of hormone dependence in human malignant disease.

The concept that leukaemias and the associated aplastic, myeloproliferative and lymphoproliferative diseases may all be different but related expressions of imbalance in the unknown physiological mechanisms stimulating and restraining haemopoietic activity offers an explanation for their interrelationship, the transitions which occur between them, and the remarkable range in clinical malignancy that they show. In some cases the imbalance may be due to a primary change in the cell, and the proliferation will then be an autonomous one, but it is not unlikely that many of the leukaemias and allied diseases occurring in man are dependent processes. If the proliferative disturbance were in fact a conditioned one, a possible explanation could be offered for the dramatic remissions occasionally observed to occur in acute leukaemias either spontaneously or as a result of transfusion or the administration of steroid hormones, since it is easier to visualize the controlling mechanisms temporarily returning to normal than to imagine an autonomously malignant cell line losing its malignant characters for a short period and then regaining them. The idea of conditioned proliferation would also conform well with the lack of specifically malignant cytological characteristics in leukaemic cells and the documented

reports of normal maturation of some leukaemic cells *in vitro*. Moreover, conditioned and autonomous processes are not mutually exclusive. Just as conditioned tumours produced experimentally may eventually become autonomous if growth is long enough sustained, so dependent leukaemias may become autonomous. The fact that transmissible animal leukaemias are usually clearly autonomous, except for those due to an infecting virus where transmission can be achieved with cell-free filtrates, does not therefore militate against the possible dependency of non-transmissible animal leukaemias and of leukaemias in man.

We may therefore conclude that the leukaemias are proliferative diseases falling within the general category of neoplasia in its widest sense, but very possibly due to an externally determined imbalance between the forces controlling leucopoiesis, though in some cases the proliferation may become autonomous. In so far as the hyperplasia may be conditioned, hope exists that study of the controlling mechanisms might eventually offer a completely satisfactory approach to therapy, by enabling the imbalance to be permanently corrected. Such a solution to the problem of leukaemia is not an impossible one.

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